The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed R. Mahoney

Dated 20 August 2003

Patents Form 1/77

Patents Act 1977
(Rule 16)

The Patent Office

095EP02 E746663-1 D02973
P01/7700 0.00-0220822.1

THE PATENT OFFICE
A
- 9 SEP 2002
RECEIVED BY FAX

Request for grant f a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office
Cardiff Road
Newport
South Wales
NP9 1RH

1. Your reference — JHC/P041082GB1

2. Patent application number *(The Patent Office will fill in this part)* — 09 SEP 2002 — 0220822.1

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

   Trigen Limited
   20 St James's Street
   LONDON
   SW1A 1ES

   07516081001

   Patents ADP number *(if you know it)*

   If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. Title of the invention — Boropeptides

5. Name of your agent *(if you have one)* — Harrison Goddard Foote

   "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

   Belgrave Hall
   Belgrave Street
   Leeds
   LS2 8DD

   Patents ADP number *(if you know it)* — 14571001 — 07631310002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

   a) any applicant named in part 3 is not an inventor, or
   b) there is an inventor who is not named as an applicant, or
   c) any named applicant is a corporate body.
   See note (d))

   Yes

Patents Form 1/77

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	55 ✓
Claim(s)	10 ✓
Abstract	—
Drawing(s)	—

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this applicati n.

Signature — Date 9 September 2002

12. Name and daytime telephone number of person to contact in the United Kingdom — Jonathan Couchman — 0113 233 0100

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

TITLE OF THE INVENTION

PEPTIDE BORONIC ACIDS

BACKGROUND OF THE INVENTION

The present invention relates to novel products which are pharmaceutically acceptable and/or useful for pharmaceutical purposes. The products comprise boropeptides and are useful for treating arterial thrombosis. The invention includes other aspects, in particular methods of using the boropeptides.

Thrombosis

Hemostasis is the normal physiological process in which bleeding from an injured blood vessel is arrested. It is a dynamic and complex process in which proteolytic enzymes such as thrombin play a key role. Blood coagulation may occur through either of two cascades of zymogen activations, the extrinsic and intrinsic pathways of the coagulation cascade. Factor VIIa in the extrinsic pathway, and Factor IXa in the intrinsic pathway are important determinants of the activation of factor X to factor Xa, which itself catalyzes the activation of prothrombin to thrombin. The last protease in each pathway is thrombin, which acts to hydrolyze four small peptides (two FpA and two FpB) from each molecule of fibrinogen, thus deprotecting its polymerization sites. Once formed, the linear fibrin polymers may be cross-linked by factor XIIIa, which is itself activated by thrombin. In addition, thrombin is a potent activator of platelets, upon which it acts at specific receptors. Thrombin activation of platelets leads to aggregation of the cells and secretion of additional factors that further accelerate the creation of a hemostatic plug. Thrombin also potentiates its own production by the activation of factors V and VIII (see Hemker and Beguin in: Jolles, et. al., "Biology and Pathology of Platelet Vessel Wall Interactions," pp. 219-26 (1986), Crawford and Scrutton in: Bloom and Thomas, "Haemostasis and Thrombosis," pp. 47-77, (1987), Bevers, et. al., *Eur. J. Biochem.* **1982**, 122, 429-36, Mann, *Trends Biochem. Sci.* **1987**, 12, 229-33).

Platelets thus play two important roles in normal hemostasis. First, by aggregating, they consitute the intitial hemostatic plug which immediately curtails bleeding from broken blood vessels. Secondly, the platelet surface can become activated and potentiate blood clotting, a property referred to as platelet procoagulant activity. This may be observed as an increase in the rate of activation of prothrombin by factor Xa in the presence of factor Va and Ca^{2+}, referred to as the prothrombinase reaction. Normally, there are few (if any) clotting factors on the surface of unstimulated platelets but, when platelets are activated, negatively charged phospholipids (phosphatidylserine and phospatidylinositol) that are normally on the cytoplasmic side of the membrane become available and provide a surface on which two steps of the

coagulation sequence occur. The phospholipid on the surface of activated platelets profoundly accelerates the reactions leading to the formation of thrombin, so that thrombin can be generated at a rate faster than its neutralisation by antithrombin III. The reactions that occur on the platelet surfaces are not easily inhibited by the natural anticoagulants in blood such as antithrombin III, either with or without heparin. (See Kelton and Hirsch in : Bloom and Thomas, "Haemostasis and Thrombosis," pp. 737-760, (1981); Mustard et al in : Bloom and Thomas, "Haemostasis and Thrombosis," pp. 503526, (1981); Goodwin et al; *Biochem. J.* **1995**, *308*, 15-21).

A thrombus can be considered as an abnormal product of a normal mechanism and can be defined as a mass or deposit formed from blood constituents on a surface of the cardiovascular system, for example of the heart or a blood vessel. Thrombosis can be regarded as the pathological condition wherein improper activity of the hemostatic mechanism results in intravascular thrombus formation. Three basic types of thrombi are recognised:

- the white thrombus which is usually seen in arteries and consists chiefly of platelets;
- the red thrombus which is found in veins and is composed predominantly of fibrin and red cells;
- the mixed thrombus which is composed of components of both white and red thrombi.

The composition of thrombi is influenced by the velocity of blood flow at their sites of formation. In general white platelet-rich thrombi form in high flow systems, while red coagulation thrombi form in regions of stasis. The high shear rate in arteries prevents the accumulation of coagulation intermediates on the arterial side of the circulation: only platelets have the capacity to form thrombi binding to the area of damage via von Willebrand factor. Such thrombi composed only of platelets are not stable and disperse. If the stimulus is strong then the thrombi will form again and then disperse continually until the stimulus has diminished. For the thrombus to stabilise, fibrin must form. In this respect, small amounts of thrombin can accumulate within the platelet thrombus and activate factor Va and stimulate the platelet procoagulant activity. These two events lead to an overall increase in the rate of activation of prothrombin by factor Xa of 300,000 fold. Fibrin deposition stabilises the platelet thrombus. Thrombin inhibitors are not clinically effective at inhibiting stimulation of platelet procoagulant activity. Accordingly, a therapeutic agent which inhibits platelet procoagulant activity would be useful for treating or preventing arterial thrombotic conditions

On the venous side of circulation, the thrombus is comprised of fibrin: thrombin can accumulate because of the slower flow on the venous side and platelets play only a minor role.

Thrombosis is thus not considered to be a single indication but, rather, is a class of indications embracing distinct sub-classes for which differing therapeutic agents and/or protocols may be

appropriate. Thus, regulatory authorities treat disorders such as, for example, deep vein thrombosis, cerebrovascular arterial thrombosis and pulmonary embolism as distinct indications for the purposes of licensing medicines. Two main sub-classes of thrombosis are arterial thrombosis and venous thrombosis. Arterial thrombosis includes such specific disorders as acute coronary syndromes [for example acute myocardial infarction (heart attack, caused by thrombosis in a coronary artery)], cerebrovascular arterial thrombosis (stroke, caused by thrombosis in the cerebrovascular arterial system) and peripheral arterial thrombosis. Examples of conditions caused by venous thrombosis are deep vein thrombosis and pulmonary embolism.

The management of thrombosis commonly involves the use of thrombolytic agents in combination with anticoagulants and antiplatelet drugs (inhibitors of platelet aggregation) to lyse the newly formed clot and to control future thrombogenesis. Anticoagulants are used also in the treatment of patients thought susceptible to thrombosis.

Currently, two of the most effective classes of drugs in clinical use as anticoagulants are the heparins and the vitamin K antagonists. The heparins are ill-defined mixtures of sulfated polysaccharides that bind to, and thus potentiate the action of antithrombin III. Antithrombin III is a naturally occurring inhibitor of the activated clotting factors IXa, Xa, XIa, thrombin and probably XIIa (see Jaques, *Pharmacol. Rev.* **1980**, 31, pp. 99-166). The vitamin K antagonists, of which warfarin is the most well-known example, act indirectly by inhibiting the post-ribosomal carboxylations of the vitamin K dependent coagulation factors II, VII, IX and X (see Hirsch, *Semin. Thromb. Hemostasis* **1986**, 12, 1-11). While effective therapies for the treatment of thrombosis, heparins and vitamin K antagonists have the unfortunate side effects of bleeding, heparin-induced thrombocytopenia (in the case of heparin) and marked interpatient variability, resulting in a small and unpredictable therapeutic safety margin.

The use of direct acting inhibitors of thrombin and other serine protease enzymes of the coagulation system is expected to alleviate these problems. To that end, a wide variety of serine protease inhibitors have been tested, including boropeptides, i.e. peptides containing a boronic acid analogue of an N-acyl-α-amino acid. Shenvi (EP-A-145441 and US 4499082) disclosed that certain peptides containing an α-aminoboronic acid were effective inhibitors of elastase and has been followed by numerous patent publications relating to boropeptide inhibitors of serine proteases.

Claeson et al (US 5574014 and others) and Kakkar et al (WO 92/07869 and family members including US 5648338) disclose thrombin inhibitors having a neutral C-terminal side chain, for example an alkoxyalkyl side chain. The Claeson et al and Kakkar et al patent families disclose boronate esters containing the amino acid sequence D-Phe-Pro-BoroMpg [(R)-Phe-Pro-BoroMpg], which are highly specific inhibitors of thrombin (Deadman et al., *J. Med. Chem.* **1995**, *38*, 1511-

1522; Elgendy et al *Adv. Exp. Med. Biol. (USA)* **1993**, *340*, 173-178). Of these compounds may be mentioned in particular Cbz-(R)-Phe-Pro-BoroMpg-OPinacol (also known as TRI50b).

The aforementioned US patents (US 5574014 and US 5648338), are incorporated herein by reference.

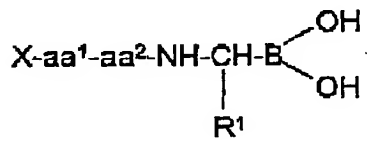
Whilst direct acting thrombin inhibitors have been found useful for the treatment of patients susceptible to or suffering from venous thrombosis, the same is not true of arterial thrombosis. It is necessary to raise the dosage of thrombin-inhibitors used in the treatment of venous thrombosis by many times in order to treat arterial thrombosis. Such raised dosages typically cause bleeding, which makes direct acting thrombin inhibitors unsuitable for treating arterial thrombosis. Heparin, which primarily acts as a thrombin inhibitor, is also unsuitable to treat arterial thrombosis.

It would be desirable to provide an additional anti-thrombotic drug which is capable of inhibiting arterial thrombosis whilst greatly reducing the incidence of serious bleeding problems and desirably without any, or any significant, bleeding in the vast majority of patients. As a potential reference value for unacceptable bleeding risk, it can be mentioned that, in the case of heparin, it is generally accepted that a dosage which leads to an increase in APTT from the normal control value of 40s to about 80s to 120s is appropriate for reducing the risk of thrombosis without creating an unacceptable risk of bleeding, whereas an increase in APTT to 200s or more is unacceptable because of the bleeding risk. (During clinical trials of antithrombotics, the tendency to cause excessive bleeding is monitored from the fall in haemoglobin, the number of units of transfusion blood used and also by noting evidence of overt bleeding. It is not usual to measure bleeding times which would be considered unethical. In the case of heparin , although it is true to say that bleeding occurs at high dosages (this often happens inadvertently through the administration of a set dose), the bleeding risk does not follow the clotting times absolutely).

BRIEF SUMMARY OF THE INVENTION

The invention provides products useful *inter alia* for inhibiting platelet procoagulant activity. The invention provides products useful *inter alia* for treating arterial thrombosis by therapy or prophylaxis.

The present invention provides a salt of a peptide boronic acid of formula (I):



(I)

where:

X is H (to form NH_2) or an amino-protecting group;

aa^1 is Phe, Dpa or a wholly or partially hydrogenated analogue thereof;

aa^2 is an imino acid having from 4 to 6 ring members; and

R^1 is a group of the formula $-(CH_2)_m-W$, where m is 2, 3 or 4 and W is –OH, –OMe, –OEt or halogen (F, Cl, Br or I).

There is a debate in the literature as to whether boronates in aqueous solution form the 'trigonal' $B(OH)_2$ or 'tetrahedral' $B(OH)_3^-$ boron species, but NMR evidence seems to indicate that at a pH below the first pKa of the boronic acid the main boron species is the neutral $B(OH)_2$. In the duodenum the pH is likely to be between 6 and 7, so the trigonal species is likely to be predominant here. In any event, the symbol $-B(OH)_2$ includes tetrahedral as well as trigonal boron species.

The invention includes also oral formulations of the salts of the invention.

According to a further aspect of the present invention, there is provided a method of treating arterial thrombosis by prophylaxis or therapy, comprising administering to a mammal, especially a human, suffering from, or susceptible to, arterial thrombosis a therapeutically effective amount of a salt of a peptide boronic acid of formula (I).

The anti-arterial thrombotic activity of the compounds is considered to comprise inhibition of platelet procoagulant activity. The invention accordingly provides a method of inhibiting platelet procoagulant activity, comprising administering to a mammal, especially a human, at risk of, or suffering from, arterial thrombosis a therapeutically effective amount of a salt of a peptide boronic acid of formula (I).

The invention also provides the use of a salt of a peptide boronic acid of formula (I) for the manufacture of a medicament for treating arterial thrombosis. Further included is the use of a salt of a peptide boronic acid of formula (I) for the manufacture of a medicament for inhibiting platelet procoagulant activity.

Other aspects of the invention reside in: an agent for the treatment of arterial thrombosis which comprises as an active ingredient a salt of a peptide boronic acid of formula (I); and an agent for inhibiting platelet procoagulant activity which comprises as an active ingredient a salt of a peptide boronic acid of formula (I).

The salts of the invention are obtainable by reaction of the acid (I) with a strong base. The invention thus provides also products obtainable by reaction of an acid (I) with a base able to form a salt with it and the therapeutic, including prophylactic, use of such products.

The invention includes a method for preparing the salts from the corresponding peptide boronic acid as an intermediate, as well as the intermediate peptide boronic acid and a method for preparing it.

Further aspects and embodiments of the invention are set forth in the following description and claims.

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of the words, for example "comprising" and "comprises", mean "including but not limited to", and are not intended to (and do not) exclude other moieties, additives, components, integers or steps.

DETAILED DESCRIPTION OF THE INVENTION

Glossary

The following terms and abbreviations are used in this specification:

α-Aminoboronic acid or Boro(aa) refers to an amino acid in which the CO_2 group has been replaced by BO_2

Arg – arginine

Cbz – benzyloxycarbonyl

Cha – cyclohexylalanine

Dcha – dicyclohexylalanine

Dpa – diphenylalanine

Lys – lysine

Mpg – 3-methoxypropylglycyl

Phe – phenylalanine

Pinac = Pinacol – 2,3-dimethyl-2,3-butanediol

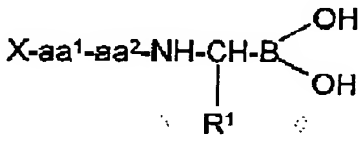
(+)-Pinanediol boronate – 1a,7,7-trimethyl-[1aS-(1aa, 4a, 6a, 5aa)]-4,6-methano-1,2-benzodioxaborole

Pro – proline

Thr – thrombin

The Compounds

The products of the invention comprise salts of a peptide boronic acid of formula (I):

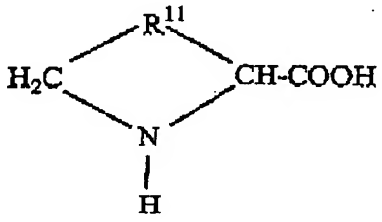


(I)

X is a moiety bonded to the N-terminal amino group and may be H to form NH_2. The identity of X is not critical to the invention but as a preferred example there may be mentioned benzyloxycarbonyl.

aa^1 is Phe, Dpa or a wholly or partially hydrogenated analogue thereof. The hydrogenated analogues are Cha and D-Dcha.

A preferred class of products comprises those in which aa^2 is a residue of an imino acid of formula (III)



(III),

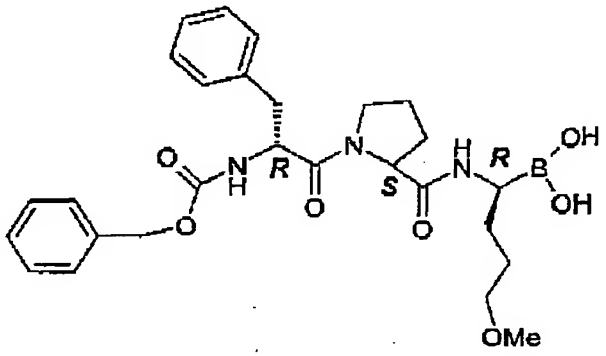
where R^{11} is $-CH_2-$, CH_2-CH_2-, $-S-CH_2-$, $-S-C(CH_3)_2-$ or $-CH_2-CH_2-CH_2-$, which group when the ring is 5 or 6-membered is optionally substituted at one or more $-CH_2-$ groups by from 1 to 3 C_1-C_3 alkyl groups. Of these, azetidine-2-carboxylic acid, especially (s)-azetidine-2-carboxylic acid, and more particularly proline are preferred.

It will be appreciated from the above that a very preferred class of products consists of those in which aa^1-aa^2 is Phe-Pro. In another preferred class, aa^1-aa^2 is Dpa-Pro. In other products, aa^1-aa^2 is Cha-Pro or Dcha-Pro. Of course, the invention includes corresponding product classes in which Pro is replaced by (s)-azetidine-2-carboxylic acid.

R^1 is a group of the formula $-(CH_2)_m-W$. Integer m is 2, 3 or 4 and W is –OH, –OMe, –OEt or halogen (F, Cl, I or, preferably, Br). The most preferred W groups are –OMe and –OEt, especially –OMe. It is preferred that m is 3 for all W groups and, indeed, for all compounds of the invention. Particularly preferred R^1 groups are 2-bromoethyl, 2-chloroethyl, 2-methoxyethyl, 4-bromoebutyl, 4-chlorobutyl, 4-methoxybutyl and, especially, 3-bromopropyl, 3-chloropropyl and 3-methoxypropyl. Most preferably, R^1 is 3-methoxypropyl. 2-Ethoxyethyl is another preferred R^1 group.

Accordingly, a very preferred class of salts consists of those of acids of the formula X-Phe-Pro-Mpg-$B(OH)_2$, especially Cbz-Phe-Pro-Mpg-$B(OH)_2$; also preferred are analogues of these compounds in which Mpg is replaced by a residue with another of the particularly preferred R^1 groups and/or Phe is replaced by Dpa or another aa^1 residue.

The aa^1 moiety of the salt is preferably of R configuration (D-configuration). The aa^2 moiety is preferably of S configuration (L-configuration). Particularly preferred salts have aa^1 of R configuration and aa^2 of S configuration. The chiral centre –NH-CH(R^1)-B- is preferably of R configuration. It is considered that commercial formulations will have the chiral centres in RSR arrangement, as for example in the case of salts of Cbz-Phe-Pro-BoroMpg-OH:



Cbz-(R)-Phe-(S)-Pro-(R)-boroMpg-OH

The salts are obtainable by contacting an acid of formula (I) with a strong base. The invention thus contemplates products (compositions of matter) having the characteristics of a reaction product of an acid of formula (I) and a strong base. The base is pharmaceutically acceptable.

As suitable salts may be mentioned:

1. Alkali metal salts;

2. Alkaline earth metal salts, for example calcium;

3. Group III metals;

4. Salts of strongly basic organic nitrogen-containing compounds, including:

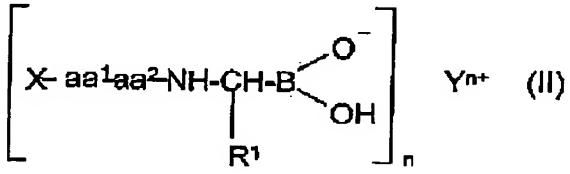
4A. Salts of guanidines and their analogues;

4B. Salts of strongly basic amine, examples of which include (i) aminosugars and (ii) other amines.

Of the above salts, the most preferred are alkali metals, especially Na and Li, and aminosugars.

The preferred salts are of the monovalent boronate (i.e. a single one of the B-OH groups is ionised) though in practice the monovalent salts may contain a very small proportion of the divalent boronate.

The invention includes therefore products (compositions of matter) which comprise salts of formula (II):



(II)

where Y^{n+} is a pharmaceutically acceptable cation obtainable from a strong base, and aa^1, aa^2, X and R^1 are as defined above.

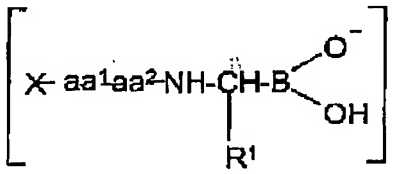
The salts preferably have a solubility of at least 10 mM, more preferably at least 20mM, when their solubility is determined as described in the examples at a dissolution of 25mg/ml. More preferably yet they have a solubility of least 50mM when their solubility is determined as described in the examples at a dissolution of 50mg/ml.

Considering the counter-ions in turn:

1. Alkali metal salts

Suitable alkali metals include lithium, sodium and potassium. All of these are remarkably soluble. Lithium and sodium are particularly preferred because of their high solubility. The lithium and particularly sodium salts are of surprisingly high solubility in relation to potassium amongst others. Sodium is most preferred. Salts containing mixtures of alkali metals are contemplated by the invention.

The invention includes products comprising salts of the formula



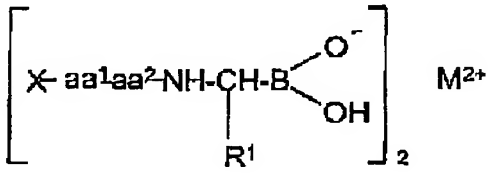
(III)

where M^+ is an alkali metal ion and aa^1, aa^2, X and R^1 are as defined above, as well as salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical M^+ group) and mixtures of such salts.

2. Alkaline earth metal salts

A suitable alkaline earth metal is calcium. As the alkaline earth metals are divalent, they are usually used in a boronic acid:metal ratio of substantially 2:1, in order to achieve the preferred monovalent boronate moiety. Salts containing mixtures of alkaline earth metals are contemplated by the invention. The alkaline earth metals are indicated to be of no more than moderate solubility and are less preferred.

The invention includes products comprising salts of the formula:

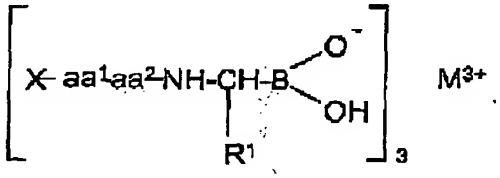


where M^{2+} is an alkaline earth metal ion and aa^1, aa^2, X and R^1 are as defined above, as well as salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical M^{2+} group) and mixtures of such salts.

3. Group III metals

Suitable Group III metals include aluminium and gallium. Salts containing mixtures of Group III metals are contemplated by the invention. The Group III metals may be of no more than moderate solubility and are less preferred.

The invention includes products comprising salts of the formula:



where M^{3+} is a Group III metal ion and aa^1, aa^2, X and R^1 are as defined above, as well as salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical M^{3+} group) and mixtures of such salts.

4. Strongly basic organic nitrogen-containing compounds

The invention includes products obtainable by (having the characteristics of a product obtained by) reaction of a peptide boronic acid as defined above and a strong organic base. Two preferred classes of organic base are described in sections 4A and 4B below. Particularly preferred are acid salts (in which one of the two boronic –OH groups is deprotonated). Most commonly, the salts contain a single type of organic counter-ion (disregarding trace contaminants) but the invention contemplates salts containing mixtures of organic counter-ions; in one sub-class, the different counter-ions all fall within the section 4A family described below or, as the case may be, in the section 4B family below; in another subclass, the salts comprise a mixture of organic counter-ions which are not all from the same family (4A or 4B).

Suitable organic bases include those with a pkb of 7 or more, e.g. 7.5 or more, for example in the region of 8 or more. Bases which are less lipophilic [e.g. have at least one polar functional group (e.g. 1, 2 or 3 such groups) for example hydroxy] are favoured; thus aminosugars are one favoured class of base.

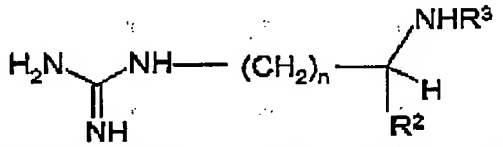
4A. Guanidines and their analogues

The guanidino compound may in principle be any soluble and pharmaceutically acceptable compound having a guanidino or a substituted guanidino group, or a substituted or unsubstituted guanidine analogue. Suitable substituents include aryl (e.g. phenyl), alkyl or alkyl interrupted by an ether or thioether linkage and, in any event, typically contain from 1 to 6 and especially 1, 2, 3, or 4 carbon atoms, as in the case of methyl or ethyl. The guanidino group may have 1, 2, 3 or 4 substituent groups but more usually has 1 or 2 substituent groups, preferably on a terminal nitrogen. One class of preferred guanidine is monoalkylated; another class is dialkylated. As guanidine analogues may be mentioned thioguanidines and 2-amino pyridines. Compounds having unsubstituted guanidino groups, for example guanidine and arginine, form one particularly preferred class.

Salts containing mixtures of guanidines are contemplated by the invention.

The guanidino compound is preferably L-arginine or an L-arginine analogue, for example D-arginine, or the D- or, preferably, L- isomers of homoarginine or agmatine [(4-aminobutyl) guanidine]. Less preferred arginine analogues are NG-nitro-L-arginine methyl ester, for example, and constrained guanidine analogues, particularly 2-amino pyrimidines, for example 2,6-quinazolinediamines such as 5,6,7,8-tetrahydro-2,6-quinazolinediamine, for example. The guanidino compound may also be a peptide, for example a dipeptide, containing arginine; one such dipeptide is L-tyrosyl-L-arginine.

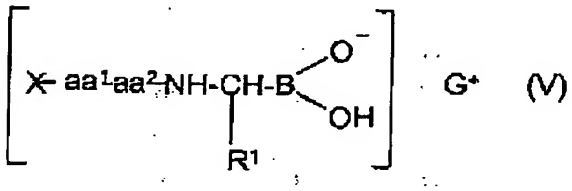
Some particularly preferred guanidino compounds are compounds of formula (IV):



(IV)

where n is from 1 to 6 and preferably at least 2, e.g. 3 or more, and preferably no more than 5. Most preferably, n is 3, 4 or 5. R^2 is H or carboxylate or derivatised carboxylate, for example to form an ester (e.g. a C_1-C_4 alkyl ester) or amide. R^3 is H, C_1-C_4 alkyl or a residue of a natural or unnatural amino acid (e.g. tyrosine). The compounds of formula (IV) are usually of L-configuration. The compounds of formula (IV) are arginine (n=3; R^2=carboxyl; R^3=H) and arginine derivatives or analogues.

The invention includes products comprising salts of the formula



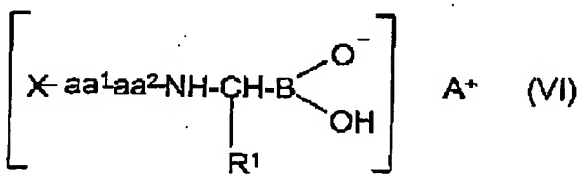
where aa^1, aa^2, X and R^1 are as defined previously and G^+ is the protonated form of a pharmaceutically acceptable organic compound comprising a guanidino group or an analogue thereof, as well as salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical G^+ group) and mixtures of such salts.

4B. Strongly basic amines

The invention includes products obtainable by (having the characteristics of a product obtained by) reaction of a peptide boronic acid as defined above and a strong organic base which is an amine. The amine may in principle be any soluble and pharmaceutically acceptable amine.

It is envisaged that a desirable class of amine includes those having polar functional groups in addition to a single amine group, as such compounds will be more hydrophilic and thus more soluble than others. Preferably, the or each additional functional group is hydroxy. Some amines have 1, 2, 3, 4, 5 or 6 additional functional groups, especially hydroxy groups. In one particularly preferred class of amines the ratio of (amino plus hydroxy groups):carbon atoms is from 1:2 to 1:1, the latter ratio being particularly preferred. These amines with one or more additional polar functional groups may be a hydrocarbon, especially an alkane, substituted by the amino group and the additional polar group(s). The amino group may be substituted or unsubstituted and, excluding amino substituents, the polar base may contain, for example, up to 10 carbon atoms; usually there are no less than three such carbon atoms, e.g. 4, 5 or 6. Aminosugars are included in this category of polar bases.

The invention includes products comprising salts of the formula



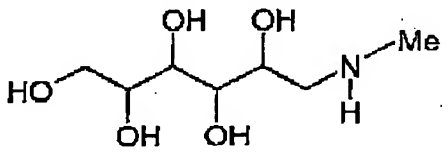
where aa^1, aa^2, X and R^1 are as defined previously and A^+ is the protonated form of a pharmaceutically acceptable amine, as well as salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical A^+ group) and mixtures of such salts. In one class of such products, A^+ is the protonated form of an amine described in section 4B(i) below; in another class A^+ is the protonated form of an amine described in 4B(ii) below

Two preferred classes of amine base are described in sections 4B(i) and 4B(ii) below. Particularly preferred are acid salts (in which one of the two boronic –OH groups is deprotonated). Most commonly, the salts contain a single type of amine counter-ion (disregarding trace contaminants) but the invention contemplates salts containing mixtures of amine counter-ions; in one sub-class, the different counter-ions all fall within the sub-section 4B(i) family described below or, as the case may be, in the sub-section 4B(ii) family below; in another subclass, the salts comprise a mixture of organic counter-ions which are not all from the same family (4B(i) or 4B(ii)).

4B(i) Aminosugars

The identity of the aminosugar is not critical to the invention. Suitable aminosugars include ring-opened sugars, especially glucamines. Cyclic aminosugars are also envisaged as useful. One class of the aminosugars is N-unsubstituted and another, preferred, class is N-substituted by one or two N-substituents (preferably one). Suitable substituents are hydrocarbyl groups, for example and without limitation containing from 1 to 12 carbon atoms; the substituents may comprise alkyl or aryl moieties or both. Preferred substituents are C_1, C_2, C_3, C_4, C_5, C_6, C_7 and C_8 alkyl groups, in particular methyl and ethyl, of which methyl is most preferred. Data indicate that aminosugars, especially N-methyl-D-glucamine, are of surprisingly high solubility.

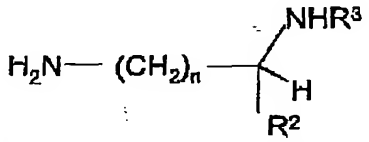
A most preferred aminosugar is N-methyl-D-glucamine:



4B(ii) Other amines

Other suitable amines include amino acids (whether naturally occurring or not) whose side chain is substituted by an amino group, especially lysine.

Some amines are compounds of formula (VII):



(VII)

where n, R^2 and R^3 are as defined in relation to formula (IV). The compounds of formula (VI) are usually of L-configuration. The compounds of formula (VI) are lysine (n=4; R^2=carboxyl; R^3=H) and lysine derivatives or analogues. A most preferred amine is L-lysine.

Other suitable amines are nitrogen-containing heterocycles. At least usually, such heterocyclic compounds are alicyclic; one class of the heterocyclic compounds is N-substituted and another, preferred, class is N-unsubstituted. The heterocycles may contain 6 ring-forming atoms, as in the cases of piperidine, piperazine and morpholine. One class of amines includes N-containing heterocycles substituted by polar substituents, especially hydroxy, e.g. 1, 2 or 3 times.

The invention therefore includes amines other than aminosugars which have one or more (e.g. 1, 2, 3, 4, 5 or 6) polar substituents, especially hydroxy, in addition to one amine group. Such compounds may have a ratio of (amino plus hydroxy groups):carbon atoms of 1:2 to 1:1, the latter ratio being particularly preferred.

The invention includes mixed salts, i.e. salts containing a mixture of boropeptide moieties and/or counterions but single salts are preferred.

The salts in solid form may contain water.

Use of the Compounds of Formula (I)

The invention provides a method for inhibiting platelet pro-coagulant activity by administering a compound of formula (I) to a mammal at risk of, or suffering from, arterial thrombosis, particularly a human patient. Also provided is the use of the compounds for the manufacture of medicaments for inhibiting platelet procoagulant activity.

The novel use of the compounds of formula (I) as inhibitors of platelet pro-coagulant activity is predicated on the observation that they are effective at inhibiting arterial, as distinct from venous, thrombosis.

Indications involving arterial thrombosis include acute coronary syndromes (especially myocardial infarction and unstable angina), cerebrovascular thrombosis and peripheral arterial occlusion and arterial thrombosis occurring as a result of atrial fibrillation, valvular heart disease, arterio-venous shunts, indwelling catheters or coronary stents. Accordingly, in another aspect the invention provides a method of treating a disease or condition selected from this group of indications, comprising administering to a mammal, especially a human patient, a compound of formula (I). The invention includes products for use in an arterial environment, e.g. a coronary stent or other arterial implant, having a coating which comprises a compound of formula (I).

The compounds of formula (I) may be used prophylactically to treat an individual believed to be susceptible to arterial thrombosis or a condition or disease involving arterial thrombosis or therapeutically (including to prevent re-occurrence of thrombosis or secondary thrombotic events).

Administration and Pharmaceutical Formulations

The compounds of formula (I) may be administered to a host. In the case of larger animals, such as humans, the compounds may be administered alone or in combination with pharmaceutically acceptable diluents, excipients or carriers. The term "pharmaceutically acceptable" includes acceptability for both human and veterinary purposes, of which acceptability for human pharmaceutical use is preferred.

The products of the invention are envisaged as having use to obtain an arterial anti-thrombogenic effect. For chronic oral use, at least for humans, the envisaged plasma concentration is at least 0.15 μg/ml and typically 0.3 to 1 μg/ml, e.g. 0.3 to 0.7 μg/ml. The chronic plasma concentrations are considered to correspond to daily oral dosages of at least 4 μmol/kg, often at least 8 μmol/kg, and in some cases 15 μmol/kg or more. It is envisaged that the daily oral dosage is unlikely to exceed 40 μmol/kg of body weight and will more typically be at least about 4 μmol/kg and typically from about 8 μmol/kg to about 30 μmol/kg of body weight, e.g. about 15 μmol/kg to about 30 μmol/kg of body weight. It is considered that the most likely daily dosage for an adult human will be from 15 μmol/kg to about 22.5 μmol/kg. The aforesaid dosages refer to the number of moles of the peptide boronate cation of the acid of formula (I) per kg of body weight.

In another aspect, the invention includes pharmaceutical formulations in unit dosage form for oral administration calculated for a 70 kg adult and thus comprising a compound of formula (I) in an amount of at least 280 μmol per unit and typically no more than 2800 μmol per unit. More usually, the mass per unit will correspond to from 550 μmol to 2100 μmol, especially 1050 μmol to 2100 μmol. The invention includes a package comprising an oral pharmaceutical formulation

containing a compound of formula (I) and instructions to take an amount of the formulation (e.g. numbers of units, for example tablets or capsules) sufficient for ingestion of at least 280 µmol of the compound and typically no more than 2800 µmol per unit. More usually, the instructions will be to take an amount sufficient for ingestion of from 550 µmol to 2100 µmol, especially 1050 µmol to 2100 µmol, of the active compound Of course, the invention includes unit dosage formulations and packages calculated on an adult weight of 60 kg.

In the case of oral administration, the compounds may be administered in a form which prevents the salt of the invention from contact with the acidic gastric juice, such as enterically coated formulations, which thus prevent release of the salt of the invention until it reaches the duodenum. Any enteric coating is suitably made of carbohydrate polymers or polyvinyl polymers, for example. Examples of enteric coating materials include, but are not limited to, cellulose acetate phthalate, cellulose acetate succinate, cellulose hydrogen phthalate, cellulose acetate trimellitate, ethyl cellulose, hydroxypropyl-methylcellulose phthalate, hydroxypropylmethylcellulose acetate succinate, carboxymethyl ethylcellulose, starch acetate phthalate, amylose acetate phthalate, polyvinyl acetate phthalate, polyvinyl butyrate phthalate, styrene-maleic acid copolymer, methyl-acrylate-methacrylic acid copolymer (MPM-05), methylacrylate-methacrylic acid-methylmethacrylate copolymer (MPM-06), and methylmethacrylate-methacrylic acid co-polymer (Eudragit® L & S). Optionally, the enteric coating contains a plasticiser. Examples of the plasticiser include, but are not limited to, triethyl citrate, triacetin, and diethyl phthalate.

The compounds of formula (I) may also be combined and/or co-administered with any antithrombotic agent with a different mechanism of action, such as the antiplatelet agents acetylsalicylic acid, ticlopidine, clopidogrel, thromboxane receptor and/or synthetase inhibitors, fibrinogen receptor antagonists, prostacyclin mimetics and phosphodiesterase inhibitors and ADP-receptor (P_2T) antagonists.

The compounds of formula (I) may further be combined and/or co-administered with thrombolytics such as tissue plasminogen activator (natural, recombinant or modified), streptokinase, urokinase, prourokinase, anisoylated plasminogen-streptokinase activator complex (APSAC), animal salivary gland plasminogen activators, and the like, in the treatment of thrombotic diseases, in particular myocardial infarction.

Typically, therefore, the products of the invention may be administered to a host to obtain a thrombin-inhibitory effect.

Actual dosage levels of active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active compound(s) that is effective to achieve

the desired therapeutic response for a particular patient, compositions, and mode of administration. The selected dosage level will depend upon the activity of the particular compound, the severity of the condition being treated and the condition and prior medical history of the patient being treated. However, it is within the skill of the art to start doses of the compound at levels lower than required for to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

According to a further aspect of the invention there is thus provided an oral pharmaceutical formulation including a product of the invention, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compound is typically mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or one or more: a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol and silicic acid; b) binders such as carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose and acacia; c) humectants such as glycerol; d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates and sodium carbonate; e) solution retarding agents such as paraffin; f) absorption accelerators such as quaternary ammonium compounds; g) wetting agents such as cetyl alcohol and glycerol monostearate; h) absorbents such as kaolin and bentonite clay and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycol, for example.

Suitably, oral formulations may contain a dissolution aid. The dissolution aid is not limited as to its identity so long as it is pharmaceutically acceptable. Examples include nonionic surface active agents, such as sucrose fatty acid esters, glycerol fatty acid esters, sorbitan fatty acid esters (e.g., sorbitan trioleate), polyethylene glycol, polyoxyethylene hydrogenated castor oil, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene alkyl ethers, methoxypolyoxyethylene alkyl ethers, polyoxyethylene alkylphenyl ethers, polyethylene glycol fatty acid esters, polyoxyethylene alkylamines, polyoxyethylene alkyl thioethers, polyoxyethylene polyoxypropylene copolymers, polyoxyethylene glycerol fatty acid esters, pentaerythritol fatty acid esters, propylene glycol monofatty acid esters, polyoxyethylene propylene glycol monofatty acid esters, polyoxyethylene sorbitol fatty acid esters, fatty acid alkylolamides, and alkylamine oxides; bile acid and salts thereof (e.g., chenodeoxycholic acid, cholic acid, deoxycholic acid, dehydrocholic acid and salts thereof, and glycine or taurine conjugate thereof); ionic surface

active agents, such as sodium laurylsulfate, fatty acid soaps, alkylsulfonates, alkylphosphates, ether phosphates, fatty acid salts of basic amino acids; triethanolamine soap, and alkyl quaternary ammonium salts; and amphoteric surface active agents, such as betaines and aminocarboxylic acid salts.

It is contemplated that the compounds of formula (I) might be formulated for oral administration using lipophilic or amphiphilic carriers or excipients, and as exemplary carriers or excipients may be mentioned non-ionic surfactants, e.g ethoxylated glycerides (suitably but not necessarily containing between 10 and 16 ethoxy groups) such as polyglycolised glycerides. Suitable polyglycolised glycerides are sold under the trade mark Gelucire®. Other contemplated carriers or excipients are poloxamers (i.e. polyoxyethylene-polyoxypropylene copolymers), preferably containing between 70% and 80% by weight of the polyoxyethylene portion. For example, a solid dispersion of the active compound in the poloxamer (e.g. poloxamer F108) may be dispersed in hydroxypropylmethylcellulose or another water-swellable polymer. The solid dispersion may contain a surfactant (e.g. sodium lauryl sulfate). The polymer dispersion may form the core of a tablet and be coated with a polymeric coating (e.g. that sold under the trade mark Eudragit)®. Suitable formulations are described in WO 99/26652 (Danbiosyst). Alternative formulations are described in WO 97/02017 and its national phase equivalents (Elan Corporation). The US national phase applications derived from these International applications are incorporated herein by reference.

The peptide boronic acid may be in finely divided solid form, for example it may be micronised.

The active compounds may also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan and mixtures thereof. Besides inert diluents, the oral compositions may also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavouring and perfuming agents. Suspensions, in addition to the active compounds, may contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan

esters, microcrystalline cellulose, aluminium metahydroxide, bentonite, agar-agar, and tragacanth and mixtures thereof.

The active compound may be given as a single dose, in multiple doses or as a sustained release formulation.

Further provided by the invention is an isolated compound which is a peptide boronic acid of formula (I). The isolated compound may be sterile. The isolated compound may be formulated into a pharmaceutical composition.

Another aspect of the invention resides in a particulate composition consisting predominantly of a peptide boronic acid of formula (I), the peptide boronic acid preferably forming at least 75% by weight of the composition, more preferably at least 85% by weight of the composition, and most preferably at least 95% by weight of the composition. The composition may be sterile. The particulate compound may undergo formulating into a pharmaceutical composition.

Also included in the invention are sterile liquid compositions consisting of, or consisting essentially of, a peptide boronic acid of formula (I) and liquid vehicle in which it is dissolved or suspended. The liquid vehicle may be an aqueous medium, e.g. water, or an alcohol, for example methanol, ethanol, isopropanol or another propanol, another alkanol or a mixture of the aforegoing. Such compositions may be processed into a pharmaceutical formulation. The compositions may be sterile.

Synthesis

1. Peptide Synthesis

The synthesis of boropeptides, including, for example, Cbz-D-Phe-Pro-BoroMpg-OPinacol is familiar to those skilled in the art and described in the prior art mentioned above, including Claeson et al (US 5574014 and others) and Kakkar et al (WO 92/07869 and family members including US 5648338). It is described also by Elgendy et al *Adv. Exp. Med. Biol. (USA)* **1993**, *340*, 173-178; Claeson,G. et al *Biochem.J.* **1993**, *290*, 309-312; Deadman et al *J. Enzyme Inhibition* **1995**, *9*, 29-41, and by Deadman et al *J. Med. Chem.* **1995**, *38*, 1511-1522.

Stereoselective synthesis with S or R configuration at the chiral B-terminal carbon may be conducted using established methodology (Elgendy et al *Tetrahedron. Lett.* **1992**, *33*, 4209-4212; WO 92/07869 and family members including US 5648338) using (+) or (—)- pinanediol as the chiral director (Matteson et al *J. Am. Chem. Soc.* **1986**, *108*, 810-819; Matteson et al *Organometallics..* **1984**, *3*, 1284-1288). Another approach is to resolve the requisite

aminoboronate intermediate (e.g. Mpg-BOPinacol) to selectively obtain the desired (R)-isomer and couple it to the dipeptide moiety (e.g. Cbz-(R)-Phe-(S)-Pro, which is the same as Cbz-D-Phe-L-Pro) which will form the remainder of the molecule.

The boropeptides are typically synthesised initially in the form of boronic acid esters, particularly esters with diols. Such diol esters may be converted to the peptide boronic acid as described next.

2. Ester to Acid Conversion

A peptide boronate ester such as Cbz-D-Phe-Pro-BoroMpg-OPinacol may be hydrolysed to form the corresponding acid, for example as described in Example 1 below, Section H.

A novel technique for converting a diol ester of a peptide boronic acid of formula (I) into the acid comprises dissolving the diol ester in an ether and particularly a dialkyl ether, reacting the thus-dissolved diol with a diolamine, for example a dialkanolamine, to form a product precipitate, recovering the precipitate, dissolving it in a polar organic solvent and reacting the thus-dissolved product with an aqueous acid to form the peptide boronic acid. The boronic acid may be recovered from the organic layer of the mixture resulting from the reaction, for example by removing the solvent, e.g. by evaporation under vacuum or distillation. The reaction between the diol ester and the diolamine may be carried out under reflux, for example.

The identity of the diol is not critical to the invention. As suitable diols may be mentioned aliphatic and aromatic compounds having hydroxy groups that are substituted on adjacent carbon atoms or on carbon atoms substituted by another carbon. That is to say, suitable diols include compounds having at least two hydroxy groups separated by at least two connecting carbon atoms in a chain or ring. A particularly preferred diol is pinacol and other exemplary diols include pinanediol (also a preferred diol), neopentylglycol, diethanolamine, 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 2,3-butanediol, 1,2-diisopropylethanediol, 5,6-decanediol and 1,2-dicyclohexylethanediol.

The alkyl groups of the dialkyl ether preferably have 1, 2, 3 or 4 carbon atoms and the alkyl groups may be the same or different. A most preferred ether is diethyl ether.

The alkyl groups of the dialkanolamine preferably have 1, 2, 3 or 4 carbon atoms and the alkyl groups may be the same or different. A most preferred dialkanolamine is diethanolamine.

The polar organic solvent is preferably $CHCl_3$.

The aqueous acid is suitably a strong inorganic acid at a pH in the region of 1; hydrochloric acid is most preferred.

After reaction with the acid, the reaction mixture is suitably washed with, for example, NH_4Cl .

A preferred procedure is as follows

1. The pinacol ester of the selected peptide boronic acid is dissolved in diethylether.
2. Diethanolamine is added and the mixture is refluxed at 40 °C.
3. The precipitated product is removed, washed (usually several times) with diethylether and dried (e.g. by evaporation under vacuum).
4. The dry product is dissolved in $CHCl_3$. Hydrochloric acid (pH 1) is added and the mixture is stirred approximately 1h at room temperature.
5. The organic layer is removed and washed with NH_4Cl solution.
6. The organic solvent is distilled off and the residual solid product is dried.

The above process results in the formation of an ester-amide of the peptide boronic acids of formula (I), especially ester-amides with diethanolamine, and such ester-amides are themselves included in the invention.

The invention provides also the use of a peptide boronic acid of formula (I) to make a salt of the invention. Included also is a method of preparing a product of the invention, comprising contacting a peptide boronic acid of formula (I) with a base capable of making such a salt.

The peptide boronic acid of formula (I) is typically of GLP or GMP quality, or in compliance with GLP (good laboratory practice) or GMP (good manufacturing practice); such acids are included in the invention.

Similarly, the acids are usually sterile and/or acceptable for pharmaceutical use, and one aspect of the invention resides in a composition of matter which is sterile or acceptable for pharmaceutical use, or both, and comprises a peptide boronic acid of formula (I). Such a composition of matter may be in particulate form or in the form of a liquid solution or dispersion.

The intermediate acid may be in isolated form and such isolated acids are included in the invention, especially isolated compounds which are a peptide boronic acid of formula (II):

$$X\text{-(R)-Phe-(S)-Pro-(R)-Mpg-B(OH)}_2 \qquad \text{(II)}$$

wherein X is H (to form NH_2) or an amino-protecting group.

One typical way of providing the intermediate acids is as a particulate composition consisting predominantly of such a peptide boronic acid, and these compositions are included in the invention. The peptide boronic acid often forms at least 75% by weight of the composition and typically at least 85% by weight of the composition, e.g. at least 95% by weight of the composition.

Another typical way of providing the intermediate acids is as a liquid composition consisting of, or consisting essentially of, a peptide boronic acid of formula (I) and a liquid vehicle in which it is dissolved or suspended. The liquid vehicle may be an aqueous medium, e.g. water, or an alcohol, for example methanol, ethanol, isopropanol or another propanol, another alkanol or a mixture of the aforegoing.

The compositions of the intermediate acids are generally sterile. The compositions may contain the peptide boronic acid in finely divided form, to facilitate further processing.

3. Salt Synthesis

The salts may be prepared by contacting the relevant peptide boronic acid with a strong base appropriate to form the desired salt. In the case of metal salts, the metal hydroxides are suitable bases (alternatively, metal carbonates might be used, for example), whilst salts with organic bases may be prepared by contacting the peptide boronic acid with the organic base itself. The preferred salts of the invention are acid salts (one -BOH proton replaced) and, to make these salts, the acid and the base, if monovalent, are usually reacted in substantially equimolar quantities; where calcium or another divalent cation is used, the usual acid:base molar ratio is substantially 2:1, whilst the usual acid: base molar ratio is substantially 3:1 for aluminium or another trivalent cation. Generally stated therefore, the usual acid base molar ratio is substantially n:1, where n is the valency of the cation of the base.

Typically, a solution of the peptide boronic acid in a water-miscible organic solvent, for example acetonitrile or an alcohol (e.g. ethanol, methanol, a propanol, especially iso-propanol, or another alkanol), is combined with an aqueous solution of the base. The acid and the base are allowed to react and the salt is recovered. The reaction is typically carried out at ambient temperature (e.g. at a temperature of from 15 to 25°C), but an elevated temperature may be used, for example up to the boiling point of the reaction mixture but more usually lower, e.g. a temperature of up to 40°C or 50°C. The reaction mixture may be allowed to stand or be agitated (usually stirred).

The time during which the acid and the base are allowed to react is not critical but it has been found desirable to maintain the reaction mixture for at least one hour. A period of from one to two hours is usually suitable but longer reaction times are included in the invention.

The salt may be recovered from the reaction mixture by any suitable method, for example evaporation, precipitation or crystallisation. In one preferred technique, the salt is recovered by evacuating the reaction mixture to dryness. The salt is preferably thereafter purified, for example by redissolving the salt before filtering the resulting solution and drying it, for example by evacuating it to dryness or freeze drying. The redissolution may be performed using water, e.g. distilled water. The salt may then be further purified, for example in order to remove residual water by further redissolution in a suitable solvent, which is advantageously ethyl acetate followed by evaporating to dryness. The purification procedure may be carried out at ambient temperature (say, 15 to 25°C), or at a modestly elevated temperature, such as e.g. a temperature not exceeding 40°C or 50°C; for example the salt may be dissolved in water and/or solvent by agitating with or without warming to, for example, 37°C.

The invention includes a method for drying the salts of the invention and other peptide boronic acid salts, comprising dissolving them in ethyl acetate and then evaporating to dryness, e.g. by evacuation.

A preferred general procedure for synthesising salts of Cbz-Phe-Pro-BoroMpg-OH is as follows:

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added the requisite base as a 0.2M solution (0.1M for a divalent cation; 0.67M for a trivalent cation) in distilled water (190ml). The resultant clear solution is allowed to react for example by being left to stand or being agitated, for a usual period, in either case, of from one to two hours. The reaction is typically carried out at ambient temperature (e.g. 15-25°C) but alternatively the temperature may be elevated (e.g. up to 30°C, 40°C or 50°C). The reaction mixture is then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in the minimum amount of distilled water necessary (200ml to 4L), typically with warming (e.g. to 30-40°C), usually for up to 2 hours. The solution is filtered, suitably through filter paper, and evacuated to dryness, again with the temperature of the solution not exceeding 37°C, or freeze dried. The resultant product is dried under vacuum overnight to normally yield a white brittle solid. If the product is present as an oil or tacky solid then it is dissolved in ethyl acetate and evacuated to dryness to produce the product as a white solid.

In variations of the aforegoing general procedure, the acetonitrile is replaced by another water-miscible organic solvent, notably an alcohol, as discussed above, especially ethanol, methanol, iso-propanol or another propanol.

Separation of Stereoisomers

The stereoisomers of a peptide boronic acid or a synthetic intermediate aminoboronate may be resolved in, for example, any known way. Accordingly, they may be resolved by chromatography (HPLC) or salt crystallisation.

Examples

The following compounds are referred to in the Examples:

TRI50b = Cbz-Phe-Pro-BoroMpg-OPinacol.
TRI50c = Cbz-Phe-Pro-BoroMpg-OH. This is the free acid of TRI50b.

It is considered that the TRI50b and TRI50c featured in the examples are at least predominantly of the most active isomer, considered to be of RSR (DLD) configuration, as discussed above.

The solubility data presented in the examples were obtained from salts made using a modification of the salt preparation process described in the examples. The modified process differs from that described in the examples in that 100mg of TRI50c was used as starting material, the product of the redissolution in water was dried by freeze drying and the filtration was carried out through a 0.2µm filter. The salts for which solubility data are presented are believed to contain about 85% of the most active isomer, considered to be of RSR configuration. When repeated with very pure active isomer salts obtained using the procedure described in the example from isomerically pure TRI50c, the solubility data were the same as those presented within experimental error or very slightly higher.

EXAMPLE 1 – SYNTHESIS OF TRI50C

A. 3-METHOXYPROPENE

1.REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

1,4-Dioxan (SPS).

Toluene, AR grade.

Allyl Alcohol.

Sodium Hydride as 60% dispersion in mineral oil. It should be a pale grey powder. Overall white colour indicates decomposition.

Dimethyl sulphate.

Magnesium sulphate dried (SLR).

Water, standard laboratory purified water is used throughout.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

1.2 PURIFICATION OF REAGENTS

All glassware used in these purification steps is heated at 140-160°C for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of dry nitrogen before use.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure.

All glassware is heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

The mechanical stirrer should be of sufficient torque to stir a viscous suspension. The stirrer arm should be fitted to the flask through a quickfit sleeve with inert oil seal.

Reaction is conducted in a three necked flask, to allow overhead stirring, inert gas purge and sodium hydride addition. A heating mantle of appropriate size is required.

3 PROCEDURE

3.1 PREPARATION

To a mechanically stirred cooled solution under nitrogen with a gas outlet and fitted with a water condenser of allyl alcohol (107.8ml, 1.59mol) and dimethylsulphate (200ml, 1.59mol, 1.eq.) in 1,4-dioxane (1L) is added, portionwise NaH (60% dispersion in mineral oil, 63.5g, 1.59mol, 1eq.). Care is taken that the reaction temperature remains at or below room temperature and the reaction is stirred until effervescence has ceased.

3.2 PURIFICATION AND WORK-UP

The slurry is stirred, carefully, into ice (1L), and extracted with toluene (3x500ml). The organic phase is heated (mantle) with a fractionation column, to distil off at atmospheric pressure the methoxypropene, b.p. 45-60°C. Heating should be observed to keep the vapour temperature in the 45-60°C range, since unreacted allyl alcohol distils at 96-98°C.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The distilled 3-methoxypropene should be checked by ^{1}H NMR spectroscopy.

B. 3-METHOXYPROPYL BORONATE CATECHOL ESTER

1 REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

Catecholborane. The appearance should be a low melting (m.p. 12°C) solid.

3-methoxypropene. The appearance should be a clear volatile liquid. It must be stored at below 4°C.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure.

A heat gun or water bath is required to prewarm the bottle of catecholborane.

All glassware must be heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

To 3-methoxypropene (120g, 1.66mol) in a 1l flask cooled in an ice bath and fitted with a condenser, is added, dropwise by dry transfer via a dropping funnel, catecholborane (199.6g, 1eq.) (which is prewarmed, if necessary, to give a liquid) and left overnight at room temperature. Careful addition of the catecholborane is necessary as the reaction can become violently exothermic.

The mixture is heated at 60-70°C for 24hrs. The mixture is allowed to cool to room temperature.

3.2 PURIFICATION AND WORK-UP

There is no purification at this stage. Used immediately.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The catechol 3-methoxypropyl boronate should be checked by ^{1}H NMR spectroscopy. ^{o}C Signals should be observed as follows:-

δ_{60}	Signal Pattern	Assignment
7.13	4H, multiplet	Ph
3.4	2H, multiplet	$\underline{CH_2}OMe$
3.39	3H, singlet	$O\underline{Me}$
1.92	2H, multiplet	$\underline{CH_2}$
1.29	2H, multiplet	$\underline{CH_2}$

Observation of other signals would be indicative of impurities

C. 3-METHOXYPROPYL BORONATE PINACOL ESTER

1 REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

Pinacol.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure.

All glassware must be heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

To catechol 3-methoxypropaneboronate (1.66mol, from section B2) is added, at 0°C, pinacol (126g, 1eq). The solution is stirred at 0°C for 1hr. Remove the ice bath and leave at room temperature overnight.

3.2 PURIFICATION AND WORK-UP

To a 3l flask containing 1.5l hexane (lab. grade, not dried) transfer the solution from 3.1. Allow the catechol to precipitate out (storage at <4°C for 1-2 hrs. facilitates this) and decant off the hexane into a 3l separating funnel. Wash the precipitate with a further 500ml of hexane and add to the first hexane solution. Wash the hexane with water (2x500ml, analytical grade). Back extract each aqueous wash with (2x500ml) hexane. Dry the hexane layer with anhydrous $MgSO_4$. Filter (glass sinter, grade four).

Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The pinacol 3-methoxypropyl boronate product should be checked by ^{1}H NMR spectroscopy ^{o}C. Signals should be observed as follows:-

δ_{400}	δ_{60}	Signal Pattern	Assignment
3.33-3.37	-	5H, multiplet	$C\underline{H_2}$-O-$C\underline{H_3}$
1.69	-	2H, multiplet	CH_2 $C\underline{H_2}$
1.24	-	12H, singlet	pinacol
0.79	-	2H, multiplet	$C\underline{H_2}B$

Due to the presence of impurities other signals will be observed also.

If impurity levels are unacceptable, distil the product (bp. 55°C/0.4mmHg, pinacol 3-methoxypropyl boronate).

D. 4-METHOXY-1-CHLOROBUTYL BORONATE PINACOL ESTER

1 REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

Dichloromethane (AR) dried/redistilled before use.

Tetrahydrofuran (AR) dried/redistilled before use.

Hexane (AR).

Lithium diisopropylamide, 2.0M in hexane/tetrahydrofuran/ethylbenzene. The reagent must be inspected before each use. It should be a clear pale red/brown solution. If it deviates from this colour or has any white precipitate it must be discarded. Store at <6°C.

Zinc chloride, 0.5M in THF.

Cyclohexane, anhydrous, 99.5%.

Benzophenone (SLR).

Sodium metal stored under paraffin oil (SLR).

Phosphorus pentoxide (SLR).

Magnesium sulphate dried (SLR).

Water, Ultra Pure grade.

Carbon tetrachloride (GLR)

Dry ice.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

1.2 PURIFICATION OF REAGENTS

All glassware used in these purification steps is heated at 140-160°C for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of argon before use.

1.2.1 Dichloromethane

Add phosphorus pentoxide to the dichloromethane at the rate of ca. 10 g per 100cm^3 and leave to stand in a stoppered flask for at least 30 minutes. Distil the dichloromethane from the phosphorus pentoxide under a stream of dry nitrogen. The purified solvent is used immediately

1.2.2 Tetrahydrofuran

The distillation apparatus is normally set up in the laboratory ready for use and will contain tetrahydrofuran over sodium containing benzophenone (ca. 0.5 g per litre) as an indicator. If necessary top up the distillation flask with more tetrahydrofuran so that it is at most two thirds full. If the colour of the solvent in the distillation flask is not blue add sodium (in oil) in small pieces, ca. 5 mm cubes until a blue colour develops. Distil the solvent from the sodium under a stream of dry nitrogen.

The purified tetrahydrofuran is used immediately and stored.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents are used for this preparation procedure.

All glassware is heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

To a solution (0.4M, in a 10l flask) of pinacol 3-methoxypropylboronate ester (150g, 0.750mol) in anhydrous cyclohexane (1250ml) and THF (625ml) (section 1.2.2) cooled to -20°C in a carbon tetrachloride/dry ice bath, is added dry DCM (section 1.2.1, 1.22eq., 58.5ml, 0.915mol). Added to this solution (with stirring, under stream of dry argon) dropwise, to maintain the temperature between -20 °C and -15 °C, is lithium diisopropylamide (1.11eq., 416ml, 0.833mol, diluted in 500ml THF) and then zinc chloride (0.5M solution in THF,1500ml) pre cooled in ice. The reaction is allowed to warm to room temperature overnight.

3.2 PURIFICATION AND WORK-UP

The reaction mixture is diluted in hexane (2l) and poured into cold 1M sulphuric acid (1l), stir for 15 mins, and then extract with hexane (2x500ml). Wash the combined extracts with saturated $NaHCO_3$ solution (1l), saturated NaCl solution (1l). Dry the combined hexane extracts with anhydrous $MgSO_4$.

Filter immediately with a grade four glass sinter.

Remove the solvent using a rotary evaporator at room temperature and with a vacuum of ca. 1 mm/Hg. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The unpurified pinacol 4-methoxy-1-chlorobutylboronate should be checked by ^1H NMR spectroscopy. Signals should be observed as follows:-

δ400	Signal Pattern	Assignment
3.47-3.38	3H, multiplet	CH_2OMe and CHB
3.34	3H, singlet	OMe
2.0-1.62	4H, multiplet	CH_2CH_2
1.27	12H, singlet	pinacol

Due to the presence of impurities other signals will be observed also.

E. 4-METHOXY-1-BIS (TRIMETHYLISILYL) AMINOBUTYL BORONATE PINACOL ESTER

1 REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

Tetrahydrofuran (AR) dried/redistilled before use.

n-Hexane SPS grade dried/redistilled before use.

Lithium bis(trimethylsilyl)amide, 1N solution in anhydrous hexane.

Water, Ultra Pure grade.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

1.2 PURIFICATION OF REAGENTS

All glassware used in these purification steps is heated at 140-160°C for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of dry nitrogen before use.

1.2.1 Tetrahydrofuran

The distillation apparatus is normally set up in the laboratory ready for use and will contain tetrahydrofuran over sodium containing benzophenone (ca. 0.5 g per litre) as an indicator. If necessary top up the distillation flask with more tetrahydrofuran so that it is at least two thirds

full. If the colour of the solvent in the distillation flask is not blue add sodium in oil in small pieces, ca. 5 mm cubes, until a blue colour develops. Distil the solvent from the sodium under a stream of dry nitrogen.

The purified tetrahydrofuran is used immediately and not stored.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure. All glassware must be heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

A 0.33M solution of pinacol 4-methoxy-1-chlorobutaneboronate (150g, 0.60mol) in THF (1810ml) is added to a 0.5M solution of lithium hexamethyldisilazane (1N in hexane, 604ml, 1eq) in THF (603ml) at -78°C (dry ice/acetone bath) giving a final concentration of boronate at 0.2M. The reaction mixture is allowed to warm slowly to room temperature and is stirred for at least 12hrs.

3.2 PURIFICATION AND WORK-UP

Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

Hexane (lab grade, 1000ml) is added to yield a precipitate which is removed by washing with water (2x750ml, analytical grade). Back extract each aqueous phase with (500ml) hexane. Dry the hexane layer with anhydrous $MgSO_4$ and filter through a grade 4 glass sinter. The organic phase is concentrated using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

The residual oil is distilled under reduced pressure to give b.p. 80-104°C, 0.1 – 0.2 mmHg pinacol 4-methoxy-1-bis(trimethylsilyl)aminobutyl boronate.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The distilled pinacol 4-methoxy-1-bis(trimethylsilyl)aminobutyl boronate should be checked by ^1H NMR spectroscopy ^0C. Signals should be observed as follows:-

δ_{400}	Signal Pattern	Assignment
3.23-3.26	5H, multiplet	$C\underline{H}_2O\ C\underline{H}_3$
2.41	1H, multiplet	$CH_2\ C\underline{H}B$
1.62	2H, multiplet	$C\underline{H}_2\ CH_2$
1.46	1H, multiplet	1H from $C\underline{H}_2$ (split H)
1.31	1H, multiplet	1H from $C\underline{H}_2$ (split H)
1.12	12H, singlet	pinacol

Due to the presence of impurities other signals will be observed also.

F. 4-METHOXY-1-AMINOBUTYL BORONATE PINACOL ESTER

1. REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

n-Hexane SPS grade dried/redistilled before use.

Chloroform (AR) dried/redistilled before use.

HCL, 4N anhydrous solution in 1,4-dioxan.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

1.2 PURIFICATION OF REAGENTS

All glassware used in these purification steps is heated at 140-160°C for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of dry nitrogen before use.

1.2.1 n-Hexane

Add calcium hydride to the n-hexane at the rate of ca. 10 g per $100cm^3$ and leave to stand in a stoppered flask for at least 30 minutes. Distil the hexane from the calcium hydride under a

stream of dry nitrogen. The purified solvent should be used immediately wherever possible but may be stored for up to 24 hours in a tightly stoppered flask.

1.2.2 Chloroform.

Add phosphorus pentoxide to the chloroform at the rate of ca. 10 g per $100cm^3$ and leave to stand in a stoppered flask for at least 30 minutes. Distil the chloroform from the phosphorus pentoxide under a stream of dry nitrogen. The purified solvent should be used immediately wherever possible but may be stored for up to 24 hours in a tightly stoppered flask.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure

All glassware is heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

To a 0.4M solution of pinacol 4-methoxy-1-bis(trimethylsilyl)aminobutane boronate (160g, 0.428mol) in dry hexane (1072ml, section 1.2.1) at -78°C (dry ice/acetone), is added HCl(4N, solution in dioxane, 322ml, 3eq.) from a measuring cylinder. The reaction is allowed to warm to room temperature overnight.

3.2 PURIFICATION AND WORK-UP

Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

Dry chloroform (2l, section 1.2.2) is added. The solution is then filtered through celite under nitrogen pressure in a closed system(grade four glass sinter). Organic phase is concentrated using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

Pinacol 4-methoxy-1-aminobutyl boronate should be checked by electrospray mass spectrometry. The signals observed should be:

Signal (AMU)	Assignment
253	[M+Na]
230	[M+]

Due to the presence of impurities other signals will be observed also.

G. Cbz-D-Phe-Pro-BoroMpg-OPinac (TRI50b)

1.REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

Tetrahydrofuran (AR) dried/redistilled before use.

n-Hexane SPS grade.

Isobutylchloroformate.

N-methylmorpholine.

Triethylamine.

Benzophenone (SLR).

Sodium Chloride (SLR).

Sodium bicarbonate (SLR).

Hydrochloric Acid (SLR).

Sodium metal stored under paraffin oil (SLR).

Magnesium sulphate dried (SLR).

Water, Ultra Pure grade.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

1.2 PURIFICATION OF REAGENTS

All glassware used in these purification steps is heated at 140-160°C for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of dry nitrogen before use.

1.2.1 Tetrahydrofuran

The distillation apparatus is normally set up in the laboratory ready for use and will contain tetrahydrofuran over sodium containing benzophenone (ca. 0.5 g per litre) as an indicator. If necessary top up the distillation flask with more tetrahydrofuran so that it is at least two thirds full. If the colour of the solvent in the distillation flask is not blue add sodium in oil in small pieces, ca. 5 mm cubes, until a blue colour develops. Distil the solvent from the sodium under a stream of dry nitrogen.

The purified tetrahydrofuran is used immediately and not stored.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure.

All glassware is heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

To a 0.5M solution of Cbz-D-Phe-Pro (0.515mol,204.5g,1eq) in THF (1042ml) is added N-methylmorpholine (56.8ml, 1eq.) and the solution cooled to -20°C (CCl_4/dry ice bath). $^iBuOCOCL$ (67ml,1eq, in 149ml THF, 3.5M) is added making sure the temperature stays in the range of -20 °C to -15°C. After 15 mins, to the mixture, is added by dry transfer a 1.36M solution of pinacol 4-methoxy-1-aminobutylboronate hydrochloride (150g, 0.57mol, 1.05eq) as a precooled solution in $CHCl_3$ (416ml), then Et_3N (75.3ml,1.05eq) is added. The reaction is allowed to warm to room temperature and stirred for at least 2hrs.

3.2 PURIFICATION AND WORK-UP

Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

The residue is dissolved in ethyl acetate (1500ml) and washed with HCl (0.2M, 2x500ml),back extract the combined HCl washes with ethyl acetate (500ml) and combine with ethyl acetate layer. Wash combined ethyl acetate with water (1000ml), back extract the water wash with 500ml of ethyl acetate combined with ethyl acetate layer, $NaHCO_3$ (saturated aqueous, 2x1000ml) and NaCl (saturated aqueous, 500ml). To the organic phase is added dried magnesium sulphate until it flocculates, the flask stoppered tightly and left to stand for at least 30 minutes. Remove the magnesium sulphate by filtration through a glass sinter, (grade four). Remove the solvent using a rotary evaporator at room temperature and with a vacuum of ca. 1 mm/Hg. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

Leave overnight on high vacuum.

The desired crude product as a foamy solid.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

3.3.1 NMR Analysis

The TRI50b should be checked by ^1H NMR spectroscopy. Signals should be observed as follows:-

δ_{400}	Signal Pattern	Assignment
7.82	1H, broad	NH
7.40-7.20	10H, multiplet	2xPh
5.7	1H, broad	NH
5.17-5.08	2H, dd, J=7.54Hz	$PhCH_2O$
4.48-4.44	2H, multiplet	Pro α-CH, Pheα-CH
3.46	1H, multiplet	Pro-C4
3.27	2H, multiplet	CH_2OMe
3.22	3H, singlet	OMe
2.99	2H, multiplet	$PhCH_2$
2.63	1H, multiplet	CHB

2.59-2.23	4H, multiplet	Pro-C3, Pro-C2
1.60	4H, multiplet	$\underline{CH_2}\underline{CH_2}$
1.20	12H, singlet	pinacol

The TRI50b should be checked by ^{13}C NMR spectroscopy. ^{0}C Signals should be observed as follows:-

δ_{400}	Signal Pattern	Assignment
171	quaternary	O-$\underline{C}$O-N
156	quaternary	CH-$\underline{C}$O-N
136	quaternary	Ph
130-126	CH	aromatics
81.5	quaternary	$\underline{C}Me_2$
73	CH_2	$\underline{C}H_2OMe$
67.26	CH_2	$PhCH_2O$
58.3	CH	Pro-αCH
57.94	CH_3	OMe
54.46	CH	Phe-αCH
46.77	CH_2	Pro-4-CH_2
38.76	CH_2	Ph$\underline{C}H_2$CH
27.84-27.4	2x CH_2	$\underline{C}H_2\underline{C}H_2CH_2OMe$
25.23-24.9	4xCH_3	pinacol, major isomer
24.07	CH_2	Pro-3- CH_2

Due to the presence of impurities other signals will be observed also.

3.3.2 HPLC Analysis

[note: a) tripeptide cannot be recovered from aqueous solution, b) Dipeptide elutes at solvent front and does not give a peak in this system]

- Column: Reverse phase C-18 ODS (octadecylsilane) 2.5µm, 150x4.6mm
- Flow: 1.5ml/min.
- Detection: UV at 225 nm
- Injection volume: 0.02ml
- Solvent A: 20% MeCN in analytical grade water.
- Solvent B: 55% MeCN in analytical grade water.

- Gradient: Linear from 20 to 90% mobile phase B over initial 15 minutes. Conditions maintained at 90% mobile phase B for a further 10 minutes. Linear to 100% B over 10mins, conditions maintained at 100% B for 5 mins then re-equilibrated to initial conditions.

Component	Rt (min)
Cbz -D-Phe-Pro-(S)-boroMpgOPinacol	16(+-1)
Cbz -D-Phe-Pro-(R)-boroMpgOPinacol	17(+-1)

Cbz-D-Phe-Pro-(R)-boroMpgOPinacol is the same chirality as Cbz-D-Phe-Pro-L-boroMpgOPinacol.

H. Cbz-D-Phe-Pro-BoroMpg-OH (TRI50c)

To a solution of TRI50b (mm 608) in acetone (1g/10ml), is added phenyl boronic acid (1.01 equivalent, mm 120) and the solution stirred by a mechanical stirrer. To the solution is slowly added ammonium hydroxide solution, (5%, pH adjusted to pH 9 by HCl, same volume as acetone). Some cloudiness may develop.

Hexane (equal volume to total acetone and ammonium hydroxide) is added and the solution stirred rapidly for four hours. Stirring is stopped and the hexane layer decanted (if an oil forms, this is kept with the aqueous layer by washing with a small volume of acetone). Hexane (same volume) is added, stirred for 10mins, decanted and repeated.

The aqueous layer is concentrated to about 1/3 volume by rotary evaporator with card-ice cold finger (water bath <35°C). Some oil may form on the side of the flask. The solution is then acidified (0.1N HCl) to pH 3 (care: do not acidify below pH 3), and extracted by EtOAc (2x same as original acetone volume). Sample can be concentrated without drying to give a foam, yield ~70%.

EXAMPLE 2 – ALTERNATIVE CONVERSION OF TRI50B TO TRI50C

1. Approximately 300 g of TRI50b were dissolved in approximately 2.5 L diethylether.
2. Approximately 54 ml diethanolamine were been added, the mixture was refluxed at 40 °C.
3. The precipitated product was removed, washed several times with diethylether and dried.
4. The dry product was dissolved in $CHCl_3$. Hydrochloric acid (pH 1) was added and the mixture was stirred approximately 1h at room temperature.
5. The organic layer was removed and washed with NH_4Cl solution.
6. The organic solvent was distilled off and the residual solid product was dried.

Typical yield: Approximately 230 g

EXAMPLE 3 - PREPARATION OF LITHIUM SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added LIOH as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 500ml distilled water necessary with light warming for about 20 minutes. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

Yield 17.89g.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)	Metal % Found (Calc.)
57.14 (61.03)	6.60 (6.64)	7.34 (7.90)	2.07 (2.03)	Li 1.26 (1.31)

EXAMPLE 4 – UV/VISIBLE SPECTRA OF LITHIUM SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. The salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \varepsilon cl$ where A is the absorbance
C is the concentration
l the path length of the UV cell
and ε is the extinction coefficient.

Extinction coefficient: 451

EXAMPLE 5 – AQUEOUS SOLUBILITY OF LITHIUM SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material. The lithium salt was comparatively soluble and so was redissolved at 50mg/ml in the same manner previously described.

Solubility when dissolved at 25mg/ml: 43mM (23 mg/ml).
Solubility when dissolved at 50mg/ml: 81mM (43 mg/ml).

EXAMPLE 6 - PREPARATION OF SODIUM SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added NaOH as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 500ml distilled water with light warming for about 15-20 minutes. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid. The product may be present as an oil or tacky solid due to residual water, in which case it is dissolved in ethyl acetate and evacuated to dryness to produce the product as a white solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

Yield: Over 50%.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)	Metal % Found (Calc.)
59.93 (59.24)	6.47 (6.44)	7.31 (7.67)	1.91 (1.98)	Na 3.81 (4.20)

EXAMPLE 7 – UV/VISIBLE SPECTRA OF SODIUM SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. The salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of

calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \varepsilon cl$ where A is the absorbance

C is the concentration

l the path length of the UV cell

and ε is the extinction coefficient.

Extinction coefficient: 415.

EXAMPLE 8 – AQUEOUS SOLUBILITY OF SODIUM SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material. The sodium salt was comparatively soluble and so was redissolved at 50mg/ml in the same manner previously described.

Solubility when dissolved at 25mg/ml: 44mM (25 mg/ml).
Solubility when dissolved at 50mg/ml: 90mM (50 mg/ml).

EXAMPLE 9 - PREPARATION OF POTASSIUM SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added KOH as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 1L distilled water with warming to 37°C for about 2 hours. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid.
Yield: 14.45 mg.

The salt was then dried under vacuum over silica to constant weight (72 h).

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)	Metal % Found (Calc.)

54.84 (57.55)	6.25 (6.26)	7.02 (7.45)	2.01 (1.92)	K 4.29 (6.94)

EXAMPLE 10 – UV/VISIBLE SPECTRA OF POTASSIUM SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \varepsilon cl$ where A is the absorbance

C is the concentration

l the path length of the UV cell

and ε is the extinction coefficient.

Extinction coefficient: 438.

EXAMPLE 11 – AQUEOUS SOLUBILITY OF POTASSIUM SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material.

Solubility when dissolved at 25mg/ml: 29mM (16 mg/ml).

EXAMPLE 12 - PREPARATION OF CALCIUM SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added $Ca(OH)_2$ as a 0.1M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant product is a white brittle solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

Yield: 17.69g.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)	Metal % Found (Calc.)
55.08 (59.27)	6.43 (6.48)	7.08 (7.71)	2.01 (1.99)	Ca 3.65 (3.68)

EXAMPLE 13 – UV/VISIBLE SPECTRA OF CALCIUM SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \varepsilon c l$ where A is the absorbance

C is the concentration

l the path length of the UV cell

and ε is the extinction coefficient.

Extinction coefficient: 955.

EXAMPLE 14 – AQUEOUS SOLUBILITY OF CALCIUM SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material.

Solubility when dissolved at 25mg/ml: 5mM (5 mg/ml).

EXAMPLE 15 - PREPARATION OF ARGININE SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added arginine as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 2L distilled water with warming to 37°C for 2 hours. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the

solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

Yield: 10.54g.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)
52.47 (56.65)	7.12 (7.20)	15.25 (14.01)	1.52 (1.54)

EXAMPLE 16 – UV/VISIBLE SPECTRA OF ARGININE SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \varepsilon cl$ where A is the absorbance
C is the concentration
l the path length of the UV cell
and ε is the extinction coefficient.

Extinction coefficient: 406.

EXAMPLE 17 – AQUEOUS SOLUBILITY OF ARGININE SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material.

Solubility when dissolved at 25mg/ml: 14mM (10 mg/ml).

EXAMPLE 18 - PREPARATION OF LYSINE SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added L-lysine as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 3L distilled water with warming to 37°C for 2 hours. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid. The product may be present as an oil or tacky solid (due to residual water), in which case it is then dissolved in ethyl acetate and evacuated to dryness to produce the product as a white solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

Yield: 17.89.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)
57.03 (59.11)	7.43 (7.36)	10.50 (10.44)	1.72 (1.61)

EXAMPLE 19 – UV/VISIBLE SPECTRA OF LYSINE SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \varepsilon cl$ where A is the absorbance
C is the concentration
l the path length of the UV cell
and ε is the extinction coefficient.

Extinction coefficient: 437.

EXAMPLE 20 – AQUEOUS SOLUBILITY OF LYSINE SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material.

Solubility when dissolved at 25mg/ml: 13mM (8.6 mg/ml).

EXAMPLE 21 - PREPARATION OF N-METHYL-D-GLUCAMINE SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added N-methyl-D-glucamine as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 500ml distilled water with light warming for about 20 minutes. The solution is filtered through filer paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C, or freeze dried. The resultant product is dried under vacuum overnight to normally yield a white brittle solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

Yield: 21.31g.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)
56.67 (56.67)	7.28 (7.41)	7.74 (7.77)	1.63 (1.50)

EXAMPLE 22 – UV/VISIBLE SPECTRA OF N-METHYL-D-GLUCAMINE SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \varepsilon cl$ where A is the absorbance

C is the concentration

l the path length of the UV cell

and ε is the extinction coefficient.

Extinction coefficient: 433.

EXAMPLE 23 – AQUEOUS SOLUBILITY OF N-METHYL-D-GLUCAMINE SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt was observed to fully dissolve. The salt was comparatively soluble and so was redissolved at 50mg/ml in the same manner previously described.

Solubility when dissolved at 25mg/ml: 35mM (25 mg/ml).
Solubility when dissolved at 50mg/ml: 70mM (50 mg/ml).

EXAMPLE 24 – ALTERNATIVE PREPARATION OF ARGININE SALT OF TRI50C

The arginine salt is formed simply by adding a slight molar excess of L-arginine to a solution of 0.2-0.3mmol of TRI50c in 10ml of ethyl acetate. The solvent is evaporated after one hour, and the residue is triturated twice with hexane to remove excess arginine.

EXAMPLE 25 – SEPARATION OF DIASTEREOMERS

The R-Mpg and S-Mpg isomers of TRI50b and TRI50c are separated chromatographically as summarised below.

A solution of 5gm/ml of TRI50b in acetonitrile is prepared and 10 µL is injected to a Lichrosphere™ cyano column and eluted with a gradient of n-hexane and tetrahydrofuran with monitoring at 206nM. Analysis of the UV chromatogram indicates TRI50b isomer I ('R' configuration at α-aminoboronate centre) elutes at (retention time) Rt 11.1 minutes; TRI50b isomer II ('S' configuration at α-aminoboronate centre) elutes at Rt 13.7minutes.

Following the same procedure, TRI50c isomer I ('R' configuration at α-aminoboronate centre) elutes at (retention time) Rt 21.2 minutes; TRI50b isomer II ('S' configuration at α-aminoboronate centre) elutes at Rt 22.2 minutes.

Conditions:

Column: Lichrosphere Cyano Merck.4.6 x 250mm, 5µ.

Solvent A : n-Hexane

Solvent B THF

Gradient 0-100% B over 25 minutes

Monitor UV at 206nm

Sample concentration 5mg/ml.

The results are shown in the chromatogram of Fig 8.

The above microanalytical data show C and N amounts below calculated, suggesting the samples might have contained unremoved water.

Advantageously, at least preferred products of the invention have adequate absorption and bioavailability. For commercial utility, a product having less good solubility may be selected by virtue of a superior overall combination of properties.

EXAMPLES 26 TO 28

The following examples refer to TRI50c sodium salt.

EXAMPLE 26 - TRI50B INHIBITION OF PLATELET PROCOAGULANT ACTIVITY

Platelet pro-coagulant activity may be observed as the increase, in rate of activation of prothrombin by factor Xa in the presence of factor Va upon the addition of platelets pretreated with thrombin, caused by thrombin alone, collagen alone or a mixture of thrombin and collagen. This property is due to an increase in anionic phospholipid on the surface of the platelet with concomitant release of microvesicle from the surface. This is an essential physiological reaction and people whose platelets have reduced ability to generate procoagulant activity (Scott syndrome) show an increased tendency for bleeding.

Method:

Washed platelets are treated with either 1.15nM thrombin, 23µg/ml collagen or a mixture of both at the same concentration at 37°C. The TRI50c salt is added either for 1 minute prior to the addition of activator or immediately after the incubation with activator. Platelet procoagulant activity is determined as described previously (Goodwin C A et al, *Biochem J.* **1995** 8, 308: 15-21).

The TRI50c salt is proved to be a potent inhibitor of platelet procoagulant activity with IC_{50}'s as summarised below:

Table 1: Influence of the TRI50C salt on the induction of platelet procoagulant activity by various agonists:

Table 1

Agonist	Fold acceleration without TRI5c	IC50 plus incubation (nM)	IC50 without incubation (nM)
Thrombin	+	+	+++++
Collagen	++	++	+++
Thrombin/Collagen	++++	+	++

Key: The greater the number of crosses (+), the greater the numerical value.

Table 1 records, for example, that when platelets are treated with thrombin they caused a many-fold acceleration of the rate of activation of prothrombin in comparison with control platelets. Treatment with the TRI50C salt reduces such acceleration by half at the various TRI50 concentration levels given. The significant potency of TRI50 is evidenced by the fact that the IC_{50} values are in the nanomolar range.

The TRI50c salt does not have an effect on ADP, collagen or epinephrine induced aggregation of washed platelets.

EXAMPLE 27 - RABBIT EXTRACORPOREAL SHUNT MODEL

Introduction

The technique describes an animal model in which a platelet rich thrombus is produced. The activity of the TRI50C salt and heparin are compared.

The carotid artery and jugular vein of anaesthetised rabbits are used to create an extracorporeal circuit containing a suspended foreign surface (silk thread). Thrombus deposition is initiated by creation of high sheer stress turbulent arterial blood flow, platelet activation, followed by coagulation in the presence of thrombogenic surfaces. Histopathological studies show that the thrombus is platelet rich.

Materials and Methods

Animals:

NZW rabbits are used. The animals are allowed food and water up to the induction of anaesthesia.

Anaesthesia:

Animals are premedicated with fontanel/fluanisone (Hypnorm) 0.15 ml total by intramuscular injection. General anaesthesia is induced with methohexitone (10 mg/ml) to effect, followed by endotracheal intubation. Anaesthesia is maintained with isoflurane (1-2.0 %) carried in oxygen /nitrous oxide.

Surgical Preparation:

The animals are placed in dorsal recumbency and the ventral cervical region prepared for surgery. The left carotid artery and right jugular vein are exposed. The artery is cannulated with a large Portex® catheter (yellow gauge), cut to a suitable length. The vein is cannulated with a Silastic® catheter. The shunt comprises a 5 cm length of 'auto analyser' line (purple /white gauge). Joins to the shunt on the arterial side are made with intermediate size Silastic® tubing. The shunt is filled with saline before exposure to the circulation. The right femoral artery is cannulated for the measurement of blood pressure.

Thread Preparation and insertion:

The central section of the shunt contains a thread 3 centimetres in length. This consists of 000 gauge Gutterman sewing silk so as to give four strands with a single knot at the end. (The knot section is outside the shunt).

Blood Flow

Blood flow velocity is determined by use of 'Doppler' probes (Crystal Biotech). A silastic probe is positioned over the carotid artery at the point of insertion of the arterial catheter. Flow is recorded on a chart recorder using heat sensitive paper.

RESULTS

Table 2

TREATMENT	DOSE (APPROX)	THROMBUS WEIGHT AFTER 20 minute run	ANTITHROMBOTIC ACTIVITY
Control	N/A	+++	
TRI50c salt	10mg/kg iv	+	Active
	3.0mg/kg iv	++	Active
HEPARIN	100 u/kg iv	+++	Inactive
	300 u/kg iv	+	Active (Severe bleeding)

Key: the greater the number of crosses, the greater the weight.

Discussion

Table 2 shows that, under high arterial shear conditions, an approximate the TRI50C salt dose of 3mg/kg to 10mg/kg iv significantly inhibits thrombus formation without bleeding, whereas a heparin dose within the normal clinical range for treating venous thrombosis (100u/kg iv heparin) is ineffective. The higher dose of heparin, though active, causes severe bleeding. These results, which show the TRI50C salt effectively inhibiting arterial thrombosis without causing bleeding, are consistent with the TRI50C salt inhibiting platelet procoagulant activity. In contrast, the thrombin inhibitor heparin, when administered at an approximately equi-effective dose (in terms of inhibition of arterial thrombosis), produces the severe bleeding normal when thrombin inhibitors are used to treat arterial thrombosis.

EXAMPLE 28 – COMPARISON OF BLEEDING TIMES

The aim of the study is to compare the bleeding times of heparin with the TRI50C salt in a suitable model. It is accepted that heparin is a poor inhibitor of platelet procoagulant activity (*J. Biol. Chem.* **1978** Oct 10; 253(19):6908-16; Miletich JP, Jackson CM, Majerus PW1; *J. Clin. Invest.* **1983** May; 71(5):1383-91).

Bleeding times are determined in a rat tail bleeding model following intravenous administration of heparin and the TRI50C salt. The doses employed are chosen on the basis of their efficacy in the rat Wessler and dynamic models and are as follows:

TRI50C salt: 5 and 10 mg/kg
Heparin: 100 units/kg

MATERIALS AND METHODS

Anaesthesia

Rats are anaesthetised with sodium pentabarbitone at 60 mg/kg (2.0 ml/kg of 30 mg/ml solution by ip. injection). Supplemental anaesthetic is given ip. as required.

Surgical preparation

A jugular vein is cannulated for the administration of test compound. The trachea is also cannulated with a suitable cannula and the animals allowed to breathe 'room air' spontaneously.

Compound administration

These are given in the appropriate vehicle at 1.0 ml/kg intravenously. Heparin is administered in saline, whilst the TRI50C salt is dissolved in ethanol, and then the resultant solution added to water for injection (1 part ethanol to 5 parts water).

Technique

Two minutes following compound administration the distal 2mm of the animal's tail is sectioned with a new scalpel blade and the tail immersed in warm saline (37°C) contained in a standard 'universal' container, so that the blood stream is clearly visible. The bleeding time recording is started immediately following transection until the cessation of blood flow from the tip of the tail. A period of 30 seconds is allowed after the blood flow from the tail had stopped to ensure that bleeding does not re-commence, if bleeding does start again the recording time was continued for up to a maximum of 45 minutes.

Results

Table 3 gives a summary of the bleeding results and shows the increases above base line values.

Table 3

Summary table of bleeding results

Treatment	Bleeding time min (± SEM†)
Saline	+
Heparin 100 u/kg iv	+++++*
TRI50c salt 5 mg/kg iv	++
TRI50c salt 10 mg/kg iv	+++

*Severe bleeding in all animals, with no cessation after 40 minutes.

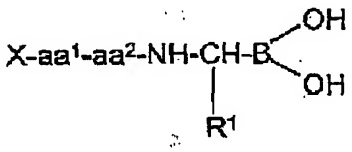
Key: the greater the number of crosses, the greater the bleeding time.

Discussion

The results show that the TRI50C salt is superior to heparin (produced less bleeding) at all doses. It should be noted that when 100 u/kg heparin is compared with 5 mg/kg TRI50c salt, heparin-treated animals bleed more extensively than those receiving TRI50c salt (heparin at a dose of 100 u/kg is a less effective inhibitor of arterial thrombosis than TRI50c salt at a dose of 3.0 mg/kg). Heparin is primarily a thrombin inhibitor and a poor inhibitor of platelet procoagulant activity; the results are therefore consistent with TRI50b exerting anti-coagulant activity by inhibition of platelet coagulant activity in addition to thrombin inhibiting activity.

CLAIMS

1. A salt of a peptide boronic acid of formula (I):



(I)

where:

X is H (to form NH_2) or an amino-protecting group;

aa^1 is Phe, Dpa or a wholly or partially hydrogenated analogue thereof;

aa^2 is an imino acid having from 4 to 6 ring members;

R^1 is a group of the formula $-(CH_2)_m-W$, where m is 2, 3 or 4 and W is –OH, –OMe, –OEt or halogen (F, Cl, Br or I).

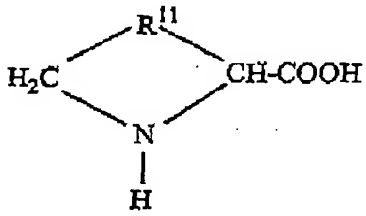
2. A salt of claim 1 wherein aa^1 is selected from Dpa, Phe, Dcha and Cha.

3. A salt of claim 1 or claim 2 wherein aa^1 is of R-configuration.

4. A salt of claim 3 wherein aa^1 is (R)-Phe (that is, D-Phe) or (R)-Dpa (that is, D-Dpa).

5. A salt of claim 3 wherein aa^1 is (R)-Phe.

6. A salt of any of claims 1 to 5 wherein aa^2 is a residue of an imino acid of formula (III)



(III),

where R^{11} is $-CH_2-$, CH_2-CH_2-, $-S-CH_2-$, $-S-C(CH_3)_2-$ or $-CH_2-CH_2-CH_2-$, which group, when the ring is 5- or 6- membered, is optionally substituted at one or more $-CH_2-$ groups by from 1 to 3 C_1-C_3 alkyl groups.

7. A salt of claim 6 wherein aa^2 is of S-configuration.

8. A salt of claim 6 wherein aa^2 is a natural proline residue.

9. A salt of claim 1, wherein aa^1-aa^2 is (R)-Phe-(S)-Pro (that is, D-Phe-L-Pro).

10. A salt of any of claims 1 to 9 wherein R^1 is 2-bromoethyl, 2-chloroethyl, 2-methoxyethyl, 3-bromopropyl, 3-chloropropyl or 3-methoxypropyl.

11. A salt of any of claims 1 to 9 wherein R^1 is 3-methoxypropyl.

12. A salt of any of claims 1 to 11 wherein X is benzyloxycarbonyl.

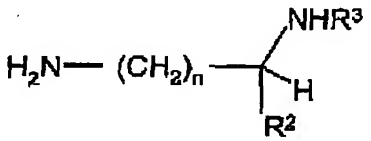
13. A salt of claim 1 which is a salt of a compound of formula (VIII):

X-(R)-Phe-(S)-Pro-(R)-Mpg-$B(OH)_2$ (VIII).

14. A salt of any of claims 1 to 13 which is a salt of the peptide boronic acid with an alkali metal, an alkaline earth metal, a Group III metal or a strongly basic organic nitrogen-containing compounds.

15. A salt of claim 14 wherein the strongly basic organic nitrogen-containing compound is a guanidine, a guanidine analogue or an amine.

16. A salt of any of claims 1 to 13 which is a salt of the peptide boronic acid with an alkali metal, an aminosugar, a guanidine or an amine of formula (VI):



(VI)

where n is from 1 to 6, R^2 is H, carboxylate or derivatised carboxylate, R^3 is H, C_1-C_4 alkyl or a residue of a natural or unnatural amino acid.

17. A salt of claim 16 which is a salt of the peptide boronic acid with an alkali metal or an amino sugar.

18. A salt of claim 17 wherein the alkali metal is sodium or lithium.

19. A salt of claim 17 or claim 18 wherein the amino sugar is a glucamine.

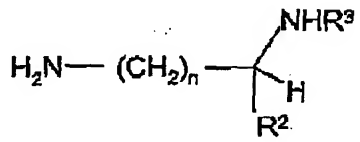
20. A salt of claim 19 wherein the glucamine is N-methyl-D-glucamine.

21. A salt of any preceding claim which is an acid salt (that is, wherein one B-OH group remains protonated).

22. A salt of any of claims 1 to 20 wherein the salt consists essentially of an acid salt (that is, wherein one B-OH group remains protonated).

23. A salt of any of claims 1 to 22 wherein the salt comprises a boronate ion derived from the peptide boronic acid and a counterion and wherein the salt consists essentially of a salt having a single type of counterion.

24. A salt of any of claims 1 to 13 which is a salt of the peptide boronic acid with a guanidine or with an amine of formula (VI):



(VI)

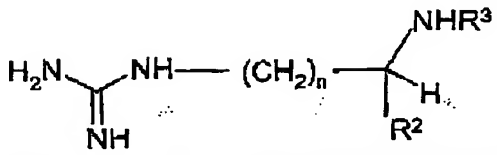
where n is from 1 to 6, R^2 is H, carboxylate or derivatised carboxylate, R^3 is H, C_1-C_4 alkyl or a residue of a natural or unnatural amino acid.

25. A salt of claim 24 which is a guanidine salt of the peptide boronic acid.

26. A salt of claim 25 which is a salt of the peptide boronic acid with L-arginine or an L-arginine analogue.

27. A salt of claim 26 wherein the L-arginine analogue is D-arginine, or the D- or L- isomers of homoarginine, agmatine [(4-aminobutyl) guanidine], NG-nitro-L-arginine methyl ester, or a 2-amino pyrimidines.

28. A salt of claim 25 which is a salt of the peptide boronic acid with a guanidine of formula (III)



(III)

where n is from 1 to 6, R^2 is H, carboxylate or derivatised carboxylate, R^3 is H, C_1-C_4 alkyl or a residue of a natural or unnatural amino acid.

29. A salt of claim 28, wherein n is 2, 3 or 4.

30. A salt of claim 28 or claim 29 where the derivatised carboxylate forms a C_1-C_4 alkyl ester or amide.

31. A salt of any of claims 28 to 30 wherein the compound of formula (III) is of L-configuration.

32. A salt of claim 25 which is an L-arginine salt of the peptide boronic acid.

33. A salt of claim 24 which is a salt of the peptide boronic acid with an amine of formula (VI).

34. A salt of claim 33, wherein n is 2, 3 or 4.

35. A salt of claim 33 or claim 34 where the derivatised carboxylate forms a C_1-C_4 alkyl ester or amide.

36. A salt of any of claims 33 to 35 wherein the compound of formula (III) is of L-configuration.

37. A salt of claim 33 which is an L-lysine salt of the peptide boronic acid.

38. A salt of any of claims 24 to 37 which is an acid salt (that is, wherein one B-OH group remains protonated).

39. A salt of any of claims 24 to 38 wherein the salt consists essentially of acid salt (that is, wherein one B-OH group remains protonated).

40. A salt of any of claims 24 to 39 wherein the salt comprises a boronate ion derived from the peptide boronic acid and a counterion and wherein the salt consists essentially of a salt having a single type of counterion.

41. A salt of any of claims 1 to 13 which is an alkali metal salt of the peptide boronic acid.

42. A salt of claim 41 wherein the alkali metal is potassium.

43. A salt of claim 42 wherein the alkali metal is sodium or lithium.

44. A salt of claim 43 wherein the alkali metal is sodium.

45. A salt of claim 43 wherein the alkali metal is lithium.

46. A salt of any of claims 41 to 45 which is an acid salt (that is, wherein one B-OH group remains protonated).

47. A salt of any of claims 41 to 45 wherein the salt consists essentially of acid salt (that is, wherein one B-OH group remains protonated).

48. A salt of any of claims 41 to 47 wherein the salt comprises a boronate ion derived from the peptide boronic acid and a counterion and wherein the salt consists essentially of a salt having a single type of counterion.

49. A salt of any of claims 1 to 13 which is an aminosugar salt of the peptide boronic acid.

50. A salt of claim 49 wherein the aminosugar is a ring-opened sugar.

51. A salt of claim 50 wherein the aminosugar is a glucamine.

52. A salt of claim 49 wherein the aminosugar is a cyclic aminosugar.

53. A salt of any of claims 49 to 52 wherein the aminosugar is N-unsubstituted.

54. A salt of any of claims 49 to 52 wherein the aminosugar is N-substituted by one or two substituents.

55. A salt of claim 54 wherein the or each substituent is a hydrocarbyl group.

56. A salt of claim 54 wherein the or each substituent is selected from the group consisting of alkyl and aryl moieties.

57. A salt of claim 56 wherein the or each substituent is selected from the group consisting of C_1, C_2, C_3, C_4, C_5, C_6, C_7 and C_8 alkyl groups

58. A salt of any of claims 54 to 57 wherein there is a single N-substituent.

59. A salt of claim 49 wherein the glucamine is N-methyl-D-glucamine.

60. A salt of any of claims 49 to 59 which is an acid salt (that is, wherein one B-OH group remains protonated).

61. A salt of any of claims 49 to 59 wherein the salt consists essentially of acid salt (that is, wherein one B-OH group remains protonated).

62. A salt of any of claims 49 to 61 wherein the salt comprises a boronate ion derived from the peptide boronic acid and a counterion and wherein the salt consists essentially of a salt having a single type of counterion.

63. A product obtainable by (having the characteristics of a product obtained by) reaction of a peptide boronic acid as defined by any of claims 1 to 13 and a base capable of forming a salt therewith.

64. A product obtainable by (having the characteristics of a product obtained by) reaction of a peptide boronic acid as defined by any of claims 1 to 13 and a base selected from the group consisting of a hydroxide of a metal as recited in any of claims 14 or 16 to 18 or with an organic nitrogen-containing compound whose pkb is 7 or more.

65. A product obtainable by (having the characteristics of a product obtained by) reaction of a peptide boronic acid as defined by any of claims 1 to 13 and a base selected from the group consisting of a organic nitrogen-containing compounds as recited in any of claims 24 to 37 or 49 to 59.

66. A product of any of claims 63 to 65 wherein the reaction comprises combining a solution of the peptide boronic acid in a water-miscible organic solvent with an aqueous solution of the base, allowing the acid and the base to react at ambient temperature (e.g. at a temperature of from 15 to 25°C), evacuating the reaction mixture to dryness, redissolving the salt in water,

filtering the resulting solution and drying it, and, if required, removing at least a portion of the residual water by further redissolution in ethyl acetate followed by evaporating to dryness.

67. A product of claim 66 wherein the acid and the base are allowed to react for at least one hour

68. A product of claim 66 or claim 67 wherein the water-miscible organic solvent is acetonitrile or an alcohol, e.g. ethanol, methanol, a propanol, especially iso-propanol, or another alkanol, or a mixture of alcohols.

69. A method for drying a peptide boronic acid salt, comprising dissolving it in ethyl acetate and then evaporating the resultant solution to dryness.

70. A pharmaceutical formulation in oral dosage form comprising a salt of any of claims 1 to 62 or a product of any of claims 63 to 68 and a pharmaceutically acceptable diluent, excipient or carrier.

71. A pharmaceutical formulation of claim 70 which is adapted to release the salt in the duodenum.

72. A pharmaceutical composition of claim 71 which is enterically coated.

73. A method of treating arterial thrombosis by prophylaxis or therapy, comprising administering to a mammal suffering from, or susceptible to, arterial thrombosis a therapeutically effective amount of a product selected from a salt of any of claims 1 to 62 and a product of any of claims 63 to 68.

74. A method of claim 73 wherein the mammal is at risk of suffering thrombosis and said product is administered orally in an amount of from 4 to 40 μmol/kg.

75. A method of claim 73 or claim 74 wherein the disease is an acute coronary syndrome.

76. A method of inhibiting platelet procoagulant activity, comprising administering to a mammal at risk of, or suffering from, arterial thrombosis a therapeutically effective amount of a product selected from a salt of any of claims 1 to 62 and a product of any of claims 63 to 68.

77. A method of claim 76 which further includes the features recited in claim 74 and/or claim 75.

78. A method of treating by way of therapy or prophylaxis an arterial disease selected from acute coronary syndromes, cerebrovascular thrombosis, peripheral arterial occlusion and arterial thrombosis resulting from atrial fibrillation, valvular heart disease, arterio-venous shunts, indwelling catheters or coronary stents, comprising administering to a mammal a therapeutically effective amount of a product selected from a salt of any of claims 1 to 62 and a product of any of claims 63 to 68.

79. A method of claim 78 which further includes the features recited in claim 74 and/or claim 75.

80. The use of a product selected from a salt of any of claims 1 to 62 and a product of any of claims 63 to 68 for the manufacture of a medicament for treating arterial thrombosis.

81. The use of a salt of any of claims 1 to 62 or a product of any of claims 63 to 68 for the manufacture of a medicament for treating by way of therapy or prophylaxis a disease selected from acute coronary syndromes, cerebrovascular thrombosis and peripheral arterial occlusion.

82. The use of claim 82 wherein the medicament is for treating an acute coronary syndrome.

83. The use, for the manufacture of a medicament for treating in a mammalian subject by way of therapy or prophylaxis a disease selected from acute coronary syndromes, cerebrovascular thrombosis, peripheral arterial occlusion and arterial thrombosis resulting from atrial fibrillation, valvular heart disease, arterio-venous shunts, indwelling catheters or coronary stents, of a salt of any of claims 1 to 62 or a product of any of claims 63 to 68.

84. The use of a salt of any of claims 1 to 62 or a product of any of claims 63 to 68 for the manufacture of a medicament for inhibiting platelet procoagulant activity.

85. The use of any of claims 80 to 84 wherein the salt or product is for oral administration in an amount of from 4 to 40 μmol/kg.

86. The use of a peptide boronic acid of formula (I) as defined in any of claims 1 to 13 as an intermediate to make a salt of any of claims 1 to 62 or a product of any of claims 63 to 68.

87. A method of preparing a salt of any of claims 1 to 62 or a product of any of claims 63 to 68, comprising contacting a peptide boronic acid of formula (I) as defined in claim 1 with a base capable of making such a salt.

88. A peptide boronic acid of formula (I) as defined in any of claims 1 to 13 when of GLP or GMP quality, or when in compliance with GLP (good laboratory practice) or GMP (good manufacturing practice).

89. A composition of matter which is sterile or acceptable for pharmaceutical use, or both, and comprises a peptide boronic acid of formula (I) as defined in any of claims 1 to 13.

90. A composition of matter of claim 89 which is in particulate form.

91. A composition of claim 89 which is in the form of a liquid solution or dispersion.

92. An isolated compound which is a peptide boronic acid of formula (II):

X-(R)-Phe-(S)-Pro-(R)-Mpg-$B(OH)_2$ (II)

wherein X is H (to form NH_2) or an amino-protecting group.

93. A compound of claim 92 wherein X is benzyloxycarbonyl.

94. A particulate composition comprising a peptide boronic acid of formula (II) as defined in claim 92 or claim 93.

95. A composition of claim 94 consisting predominantly of the peptide boronic acid.

96. A composition of claim 95 wherein the peptide boronic acid forms at least 75% by weight of the composition.

97. A composition of claim 96 wherein the peptide boronic acid forms at least 85% by weight of the composition.

98. A composition of claim 97 wherein the peptide boronic acid forms at least 95% by weight of the composition.

99. A composition of any of claims 94 to 98 which is sterile.

100. A composition of any of claims 94 to 99 wherein the peptide boronic acid is in finely divided form.

101. A liquid composition consisting of, or consisting essentially of, a peptide boronic acid of formula (I) as defined in any of claims 1 to 13 and liquid vehicle in which it is dissolved or suspended.

102. A liquid composition of claim 101 wherein the liquid vehicle is an aqueous medium, e.g. water.

103. A liquid composition of claim 101 wherein the liquid vehicle is an alcohol, for example methanol, ethanol, isopropanol or another propanol, another alkanol or a mixture of the aforegoing.

104. A liquid composition of any of claims 101 to 103 which is sterile.